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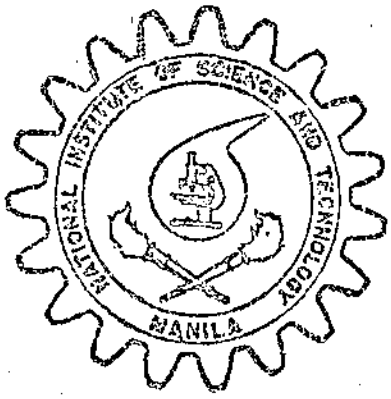

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A STUDY ON THE ISOLATION AND SCREENING OF CELLULOSE-DECOMPOSING MOLDS AS SOLUBILIZERS OF FIBROUS MATERIALS

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FIVE PLATES

ABSTRACT

Six out of 42 molds isolated from decaying organic materials which were screened for cellulose decomposing efficiency on strips of filter paper placed over mineral salts agar were selected on the basis of the following tests:

On filter paper discs impregnated with magnesium sulfate and potassium permanganate, the papers were decolorized due to the acids produced by the organisms. Tensile strength test conducted on the mold-inoculated cotton strings showed a 100 per cent decomposing efficiency after 8 days. Inoculation of bacterial membranes of *Acetobacter xylinum* resulted in its liquefaction on the 14th to the 21st day. In shaken cultures, the filter paper and coconut flour produced a loss in weight of 10 to 85 per cent after 16 days.

Analyses of coconut flour by Claudio *et al* (1966) showed that it contains 8.31-per cent crude fiber and 21-per cent protein. Although coconut flour is rich in protein, its use for human nutrition has one drawback. Its high crude fiber content provides a high percentage of indigestible material. As a food supplement for infants, growing children and the aged, it is important that it should be available in an easily digestible form.

Digestibility of the crude fiber can be improved by the action of the enzyme, cellulase, elaborated by cellulolytic micro-

organisms. This enzyme removes ligno-cellulose from coconut by-products and other plant fibers to solubilize it or cause it to become readily digestible.

The cellulose-decomposing ability of more than 400 fungus isolates was determined on test fabrics by Marsh and Bollenbacher (1949). In a separate paper, Marsh *et al* (1949) presented a review of the types of fungi which occur on cellulosic textile fibers under different exposure conditions.

Accordingly, the present paper is a report on the isolation and screening of cellulolytic molds and determination of their possibility to degrade cellulosic materials.

MATERIALS AND METHODS

Isolation of cellulose-decomposing molds.—One hundred thirty-seven samples consisting of animal manure, garden soil and decayed fruits and vegetables collected from Manila and several towns in nearby provinces were used as sources of cellulolytic molds. Isolation was done by means of the following procedure: A portion of the sample was introduced on sterile strips of filter paper placed on the surface of solidified basic salts agar,[1] a medium found to be well-adapted to the cellulose-decomposing molds and which was developed by Darby and Mandels (1954). After 2 weeks incubation at room temperature, a piece of the paper showing signs of disintegration was placed in a test tube containing sterile water. From this, pure cultures of the cellulolytic molds were obtained by the pour-plate technique of Pelczar and Reid (1959) using potato-dextrose-agar,[2] a medium recommended for the isolation of molds by Difco Laboratories (1953). Forty-two molds were isolated and maintained on strips of filter paper laid on the surface of basic salts agar slants.

[1] Formula of basic salts agar:

Ammonium nitrate (NH_4NO_3)	3.0 g
Monobasic potassium phosphate (KH_2PO_4)	2.68 g
Dibasic potassium phosphate (K_2HPO_4)	2.09 g
Magnesium sulfate ($MgSO_4.7H_2O$)	2.22 g
Yeast extract	0.1 g
Agar-agar	24.0 g
Distilled water	1,000.0 ml

[2] Formula of potato-dextrose-agar medium:

Potato, infusion from	200.0 g
Dextrose	20.0 g
Agar-agar	24.0 g
Water	1,000.0 ml

Screening of isolates and selection of cellulolytic molds adaptable for decomposition of cellulose:

1. Preliminary screening of the mold isolates for their cellulose-decomposing efficiency was conducted by inoculating 2-week-old organisms on strips of filter paper laid on the surface of solidified basic salts agar in petri dishes. The ability of the molds to cause disintegration or thinning of the paper was determined by visual examination.

This test was used as a basis of eliminating the slow decomposers and limiting the screening to only those isolates which degraded or produced thinning of the paper within a period of 14 days.

2. A test reported by Siu (1951) to determine the ability of the organism to produce acids during cellulose disintegration was done on filter paper discs impregnated with various concentrations of magnesium sulfate[3] and potassium permanganate. Filter papers cut to fit the bottom of the petri dishes were soaked in 0.2, 0.4, 0.6, 0.8 and 1.0-per cent solutions of magnesium sulfate followed by dipping in the same concentrations of potassium permanganate and drained until no more of the solutions dripped. The paper discs were dried at 45°C for 24 hours. A brown coloration was produced on the paper, the intensity of which depended on the concentration of the solutions. After sterilizing for 20 minutes at 15 pounds pressure per square inch in an autoclave, the discs were laid on the surface of solidified basic salts agar and each was inoculated at the center with the mold isolates recovered from the preliminary screening on strips of filter paper. An uninoculated disc was used as control. After incubating for 2 weeks at room temperature, the filter paper discs were observed for white spots which appeared on the brown discs as a result of the action of the acid produced on the manganese dioxide forming colorless manganese salt.

3. To determine the tensile strength of degraded cotton strings, the following experiment formulated by Siu (1951) was conducted: Commercial cotton strings, 4 ply, was cut in uniform pieces of 4 inches. These were boiled for 1 hour with 3 changes of distilled water to remove any sizing finishes which may serve as added nutrients; then dried at 45°C for 24 hours and sterilized at a pressure of 15 pounds per square inch for 20 minutes. The cotton strings were aseptically laid on the surface of solidified

[3] Magnesium sulfate was used in place of manganese sulfate which is included in the procedure of Siu (1951).

basic salts agar with a sterile forcep. The center of each cotton string was inoculated with 2-week-old culture of the mold isolates. Uninoculated strings were used as control. After 4, 8, 12, and 16 days of incubation, the strings were broken on a Scott Tensile Strength Machine and the decline in breaking strength was used as a measure of the cellulolytic activity of the organism.

4. An experiment to determine the effect of growing the cellulolytic mold isolates on cellulose membrane of *Acetobacter xylinum* was conducted in the following manner: Bacterial membranes, locally known as nata, which is synthesized by *A. xylinum* and known to be a pure form of cellulose as stated by Ram (1959) was prepared according to the method of Lapuz *et al* (1967). After the surface growth reached a thickness of 4 millimeters, the nata was picked up from the medium with the aid of a sterile forcep. The membranes were soaked for 1 to 2 days in several changes of tap water until free of acid. Following the procedure of Aschner (1937) the membranes were immersed in 5-per cent sodium hydroxide solution for 1 to 2 days, to dissolve the bacterial proteins without attacking the thick wall of cellulose which covers the individual bacteria, rinsed in tap water and transferred into dilute hydrochloric acid for 2 hours to remove traces of alkali. The membranes were washed in running water until no longer acidic and sterilized in a 1,000-ml Erlenmeyer flask containing basic salts solution.[4] The excess liquid was drained thoroughly, the membranes were spread in petri dishes and sterilized again. The dry membranes were inoculated with 2-week-old cultures of the cellulolytic mold isolates. Uninoculated membranes were used as control. After 14 days, the inoculated nata was observed for signs of liquefaction, a characteristic of cellulose-decomposing organisms.

5. To test the cellulose-decomposing capacity of the 14 selected isolates in aerated medium, the molds were inoculated in 25-ml sterilized mineral salts solution and 100-mg ground filter paper or coconut flour contained in 250-ml Erlenmeyer flasks. Uninoculated cellulose medium was used as control. Aeration was supplied by shaking the culture flasks in a reciprocal shaker. The amount of decomposed cellulose expressed as loss in weight of cellulose after subjecting to microbial attack was determined after 4, 8, 12, and 16 days incubation period. The inoculated and control samples were passed through previously tared filter pa-

[4] Basic salts solution was prepared by omitting agar-agar from the basic salts medium and whose composition is shown as footnote 3.

pers and thoroughly washed with distilled water to remove any trace of soluble material. The paper discs were dried at 100°C to constant weight. From the weight of the undecomposed residue, the quantity of digested cellulose was obtained. Computation was made on dry basis. This method was used by Siu (1951) in quantitative determination of cellulose decomposition.

RESULTS AND DISCUSSION

1. The results of the preliminary screening of 42 mold isolates for their cellulose-decomposing efficiency on strips of filter paper placed over mineral salts-agar showed that 14 of them disintegrated the paper at different periods ranging from 4 to 14 days. Isolate Nos. 45, 59, 13, 124, 5 and 71 showed thinning or disappearance of the paper in 4 to 7 days while the rest degraded the paper in 14 days. Luxuriant growth was confined on the paper, leaving the agar free of growth. After digestive action by the molds on the filter paper, only the outline of the cellulosic material was visible (Plates 1, 2, 3).

Isolate Nos. 13 (Plate 1, fig. 1a), 45, 59, and 71 (Plate 2, fig. 1a, b, c), obtained from animal manure decayed beans and soil, were observed to be similar in appearance, having compact, grayish-black growth characterized by heavy sporing. The growth of cow manure isolate, No. 5 (Plate 1, fig. 2b), was cottony white, studded with black granulelike heads at the center and produced yellow pigmentation of the medium. Canal mud isolate, No. 124 (Plate 2, fig. 2c), showed a uniform formation of coarse grayish brown heads.

Since the isolates were obtained from representative samples in the environment, we can deduce from this that cellulolytic organisms are well distributed in nature and are closely associated with decaying organic matter. The rate of decay was observed also to be accelerated in damp and warm places.

2. *Discoloration of manganese dioxide.*—In this experiment, it was observed that the growth of the molds and the bleaching effect of the acids produced by the cellulolytic organisms on the paper discs impregnated with a solution of magnesium sulfate and potassium permanganate were diminished as the concentration of the salts was increased. Table 1 shows that at 0.2 per cent, all the discs except Nos. 27 and 56 were bleached completely. At 0.4- and 0.6-per cent concentration of the salts, white spots appeared on the discs. At 0.6 and 0.8 per cent, the white

spots were greatest in the center of the discs and gradually decreased up to the periphery of the colony (Plate 4). At 0.8 and 1.0 per cent, the white spots decreased showing that high concentration of the salts could not be tolerated by the isolates.

TABLE 1.—*Bleaching effect of cellulolytic mold isolates on filter paper impregnated with various concentrations of magnesium sulfate and potassium permanganate.*

Isolate No.	0.2 Per cent	0.4 Per cent	0.6 Per cent	0.8 Per cent	1.0 Per cent
5	+++++	++++	+++	+++	+
13	+++++	++++	+++	++	+
16	+++++	+++++	++++	++++	+
24	+++++	+++++	+++++	++	+
26	+++++	+++++	++++	++++	-
27	+	+	+	-	-
41	+++++	+++++	++++	++++	+
45	+++++	++++	+++	+++	-
47	++++	++++	+	+	-
56	-	-	-	-	-
59	+++++	+++++	+++	++++	+
71	+++++	+++++	++	++	-
102	+++++	+++	+++	+++	+
124	+++++	+++++	++++	++++	+

The plus signs indicate the degree of bleaching effect of the acid produced by the mold isolates on the brown filter paper discs, which may be grouped in the following categories:

\+ ------ very weak acid producers
++ }
+++ }----- mild acid producers
+++ }
+++++ }--- strong acid producers

Basing on the results in Table 1, the isolates may be classified into three categories, the strong acid producers, those bleaching the surrounding areas completely or almost completely; the mold acid producers, those partially bleaching the area; and the very weak acid producers, those showing very little bleaching effect. It may be concluded that cellulolytic decomposition is associated with acid production and that strong cellulose decomposers are also strong acid producers. This is also substantiated

by the report of Siu (1951) wherein he listed several acids such as formic, acetic, propionic, butyric, lactic, pyruvic and succinic as one of the end products of the decomposition of cellulose by microorganisms.

3. *Determination of the tensile strength of degraded cotton strings.*—On further testing the 14 isolates on commercial cotton strings to determine the decline in breaking strength at various periods of incubation, the data in Table 2 demonstrates to us that there was slight reduction in breaking load between the 8th

TABLE 2.—*Extent of decomposition of cotton strings inoculated with cellulolytic mold isolates after different periods of incubation.*

Isolate No.	Tensile strength in kilograms and per cent of decomposition after different periods of incubation							
	4 days		8 days		12 days		16 days	
	Tensile strength	Per cent of decomposition	Tensile strength	Per cent of decomposition	Tensile strength	Per cent of decomposition	Tensile strength	Per cent of decomposition
59	0.20	94	0	100	0	100	0	100
45	0.42	88	0	100	0	100	0	100
13	0.42	88	0	100	0	100	0	100
71	0.43	88	0	100	0	100	0	100
5	0.47	87	0	100	0	100	0	100
124	0.63	83	0	100	0	100	0	100
26	2.48	33	0.40	89	0.29	92	0	100
41	2.11	43	1.16	69	0.10	97	0.04	99
47	2.33	40	1.40	62	0.49	86	0.08	98
16	2.37	33	0.83	83	0.20	94	0.1	97
102	2.84	24	1.40	62	1.20	67	0.91	76
27	3.40	8	2.44	34	1.80	51	1.76	53
24	2.60	30	2.99	20	2.98	20	1.94	48
56	3.80	0	2.64	29	2.50	33	2.26	40

The control strings broke at 3.73, 3.78, 3.80 and 3.65 kilograms with an average of 3.74. The latter was used as an index of 100 per cent.

and 12th days. Very little change also occurred between the 12th and 16th days. The tensile strength was, however, significantly lowered between the 4th and 8th days, implying that a large amount of decomposition took place during this period.

It can be seen that the tensile strength of the strings inoculated with isolate Nos. 59, 45, 71, 5, 124 and 13 was greatly reduced after 4 days, subsequently followed by a complete loss in strength after 8 days. Extensive deterioration was also exhibited by isolate Nos. 26, 16, 41 and 47 but the action was at a much slower rate. Basing on this quantitative determination of cellulose decomposition, there are 6 strong decomposers (59, 45, 71, 5, 124, 13), those producing a great loss in tensile strength in

4 days; 4 slow decomposers (26, 41, 47, 16), those exhibiting a marked loss in strength on the 8th day and the rest are weak decomposers.

4. *Cultivation of cellulose-splitting molds on membranes of A. xylinum.*—It is illustrated in Plate 5 that almost all the isolates readily decomposed the bacterial membranes. On the 3rd to the 4th day, the membranes showed signs of liquefaction. Good growth covered the entire membranes except isolate Nos. 16 and 102 wherein the growth was concentrated toward the margin of the disc. After a week of incubation, a great deal of liquid oozed from the membranes and from the 2nd to the 3rd week there was gradual shrinkage or thinning of the membrane until only thin films remained. This was a sign of solubilization of the cellulosic membrane by the molds. It was noted that while ample amount of fluid was released from all the membranes, the fastest to be solubilized were those inoculated with isolate Nos. 13, 45, 59 and 71.

This experiment demonstrated that the decomposing ability of the isolates is based on the capacity of the organisms to dissolve the cellulose medium.

5. *Cellulose decomposition in shaker flasks.*—Growth indicated by a slight turbidity and change in color of the medium was observed in all the shaker flasks after 24 to 48 hours. In Table 3, it is shown that early signs of decomposition was observed in the filter paper substrates on the 4th day of incubation as indicated by a 10 to 40 per cent loss in weight. On the 8th day about 50 per cent of the samples were decomposed by the mold isolates. Three molds, Nos. 5, 13 and 71, however, showed a significant weight loss of 60 to 67 per cent as early as 8 days. The weight of the filter paper were reduced gradually from the 4th to the 12th day presenting a loss of 60 to 85 per cent with a slight increase after the 16th day. Compared to the other isolates the weight loss of Nos. 56, 27, and 41 was considerably low.

Table 3 also shows that the loss in weight of the coconut flour between the 8th and 16th day of shaking was due to the degradation of the samples. Considering that coconut flour loses weight after a series of washings as seen in the control in Table 3 wherein 36 per cent was lost, the further loss in weight after deducting the weight of the control from the weight of the inoculated samples can therefore be attributed to the action of the microorganisms.

TABLE 3.—*Weight loss of filter paper and coconut flour substrate inoculated with different cellulolytic mold isolate after various periods of incubation.*

Isolate No.	Per cent decomposition[1] after different periods of incubation							
	4 days		8 days		12 days		16 days	
	FP	CF	FP	CF	FP	CF	FP	CF
5	41	--	67	29	79	47	84	49
13	17	--	60	32	70	41	75	44
16	20	--	55	8	56	18	65	23
24	11	--	26	--	53	19	69	21
26	30	--	59	11	74	40	80	42
27	3	--	20	--	30	10	43	14
41	32	6	43	2	49	6	55	10
45	26	--	40	15	55	23	60	26
47	17	--	60	7	67	8	62	11
56	10	--	35	17	38	28	42	12
59	25	--	34	--	66	36	68	31
71	28	--	64	24	60	25	61	39
102	11	--	43	11	66	47	70	38
124	21	--	37	--	52	22	68	30

[1]Per cent decomposition = $\frac{\text{loss in weight}}{\text{Weight of sample}}$ -- *weight of control X 100

*Average weight of controls after washing:

Filter paper (FP) = 0.100 gm
Coconut flour(CF) = 0.064 gm

Since the weight loss of the cellulosic or fibrous samples (filter paper and coconut flour) closely follow the same pattern in shaken cultures, it can be assumed that these substrates are readily degraded by cellulolytic organisms when grown under suitable cultural conditions.

Based on the results of all the screening procedures, 6 isolates (5, 13, 45, 59, 71, and 124) were selected as the most efficient cellulose decomposers.

THE IDENTITY OF THE CELLULOLYTIC MOLDS

Samples of the selected isolates were sent to Dr. R. A. Samson of Centraalbureau Voor Schimmelcultures, Baarn, Holland for identification. These isolates were identified as follows:

Nos. 13, 45, 59, 71 — *Chaetomium brasiliense* Batista & Pontual.
No. 5 — *Myrothecium verrucaria* (A. & S.) Ditm. ex Fr.
No. 124 — *Chaetomium* cf. *globosum* Kunze ex Fr.

SUMMARY

1. Forty-two mold isolates derived from animal manure, garden soil and decayed fruits and vegetables were tested in the preliminary screening for their cellulose-decomposing ability. Fourteen strains were selected based on their capability of degrading strips of filter paper laid over basic salts agar in 4 to 14 days.

2. Thirteen of the isolates (No. 56 excluded) were capable of decolorizing filter paper discs impregnated with 0.2- to 1.0-per cent solutions of magnesium sulfate and potassium permanganate, a characteristic of cellulolytic organisms wherein acids produced during digestion of cellulose cause the formation of colorless manganese salts from the brown manganese dioxide.

3. Based on tensile strength tests of inoculated cotton strings in the presence of basic salts, 6 isolates (59, 45, 71, 5, 124, 13) showed a decomposing efficiency of 83 to 97 per cent in 4 days and 100 per cent in 8 days while the rest exhibited a 40- to 100-per cent degradation after 12 to 16 days.

4. When the isolates were grown on bacterial membranes of *A. xylinum*, all the isolates produced liquefaction of the cellulosic material, a distinctive reaction of cellulose-decomposing microorganisms.

5. The weight loss of filter paper and coconut flour substrates inoculated with the different cellulolytic mold isolates and aerated in the presence of basic salts solution after 16 days ranged from 10 to 84 per cent.

ACKNOWLEDGMENT

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REFERENCES

ASCHNER, M., (1937). Cultivation of cellulose-splitting bacteria on membranes of Acetobacter xylinum. Jour. Bact. **33**: 249-252.

CLAUDIO, T. R., S. A. CAPULSO, A. L. GONZALES, F. S. DE LA FUENTE, and G. C. MANALAC (1966). Coconut Studies: II. Preliminary laboratory-scale studies on the preparation of coconut flour from granulated coconut. NIST-IRC (CRL) CS-0266.

DARBY, R. T., and G. R. MANDELS (1954). Inorganic nutrition of Myrothecium verrucaria. Mycologia **46**: 276-288.

DIFCO LABORATORIES (1953). Difco manual of dehydrated culture media and reagents for microbiological and clinical procedures. Michigan, U.S.A. Difco Lab. Inc., 350 pp.

LAPUZ, MARTINA M., E. G. GALLARDO, and M. A. PALO (1967). The nata organism — cultural requirements, characteristics and identity. Philip. Jour. Sci. **96**: 91-109.

MARSH, P. B., and K. BOLLENBACHER (1949). The fungi concerned in fiber deterioration. I. Their occurrence. Tex. Res. Jour. **19**: 313-324.

MARSH, P. B., K. BOLLENBACHER, M. L. BUTLER, and K. B. RAPER (1949). The fungi concerned in fiber deterioration. II. Their ability to decompose cellulose. Tex. Res. Jour. **19**: 462-484.

PELCZAR, MICHAEL J., JR., and ROGER D. REID (1959). Microbiology. New York: McGraw-Hill Book Co., Inc., viii + 564 pp.

RAM, VENKATA C. S. (1959). A technique for studying pH changes produced by cellulolytic fungi in cellulose substrate. Current Science **28**: 115-116.

SIU, R. G. (1951). Microbial Decomposition of Cellulose. New York: Reinhold Pub. Corp., xi + 531 pp.

ILLUSTRATIONS

[The photographs, Plates 1-5, were taken by Mr. Ricardo Marquez, technical photographer, Division of Documentation.]

PLATES 1, 2, 3

Showing degradation produced by 2-week-old cultures of the 14 cellulolytic mold isolates on strips of filter paper placed on the surface of basic salts agar and with an uninoculated strip as control.

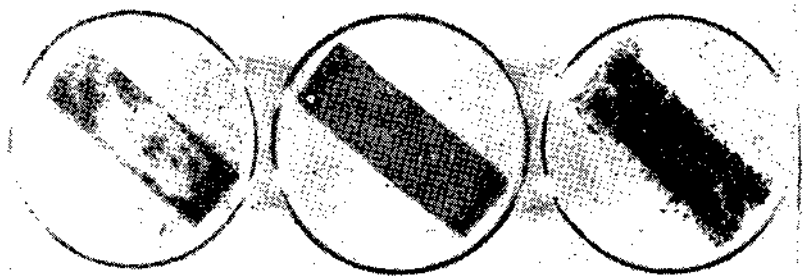
Plate 1, fig.	1	a-13	b-47	c-102
	2	a-56	b-5	c-41
Plate 2, fig.	1	a-59	b-71	c-45
	2	a-27	b-16	c-124
Plate 3		a-24	b-26	c-Original uninoculated strip

PLATE 4

Showing 2-week-old cultures of the 14 cellulolytic molds on filter paper discs impregnated with solutions of 0.6-per cent magnesium sulfate and potassium permanganate. The acids produced by the molds decolorized the brown manganese dioxide forming colorless manganese salt. The uninoculated disc was used as control.

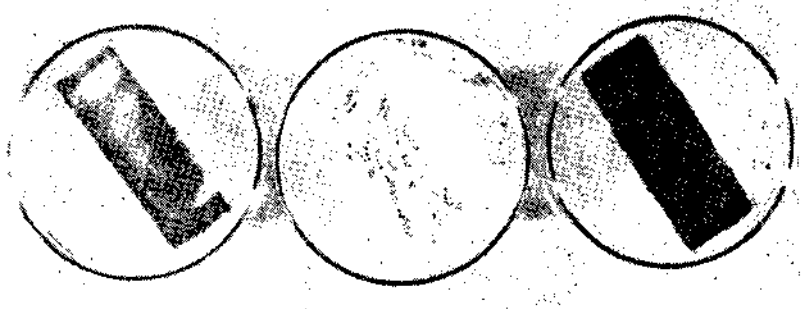
PLATE 5

Showing the liquid oozed from the cellulose membranes of *A. xylinum* and the distorted shape or shrinkage of some as a result of the solubilizing action of 2-week-old cultures of the 14 cellulolytic molds. The uninoculated membrane was used as control.



1

a *b* *c*



a *b* *c*

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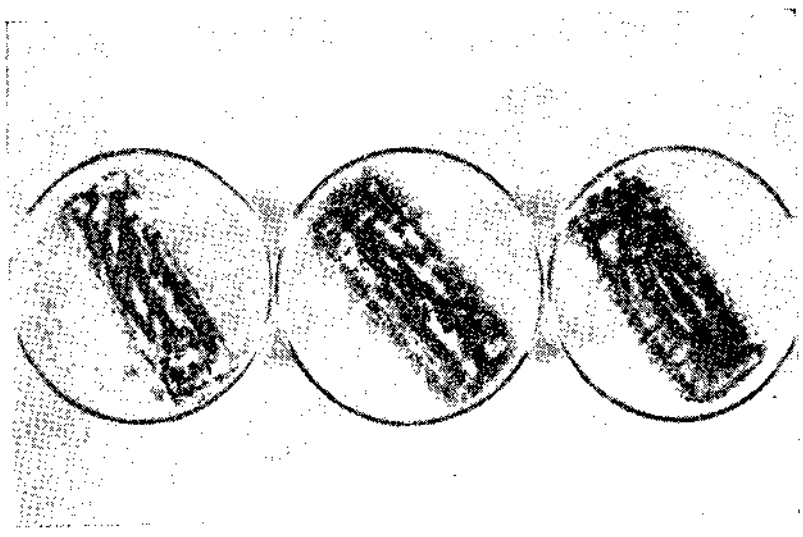
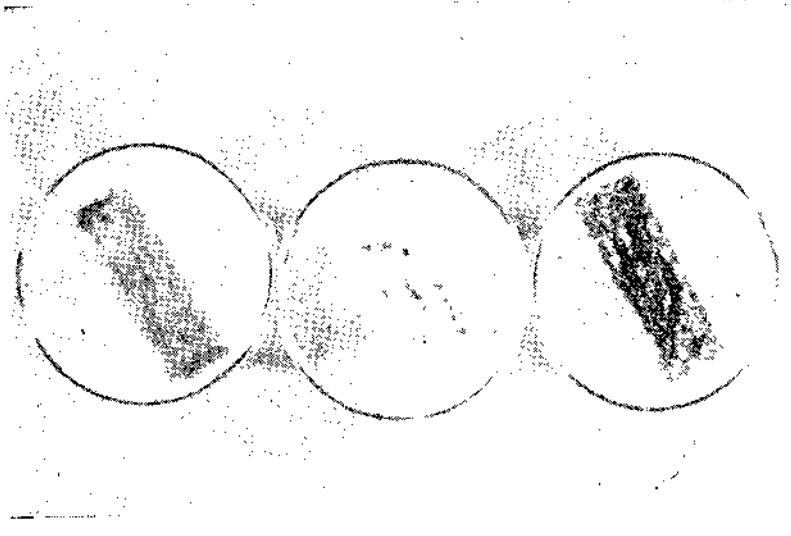
PLATE 1.



1

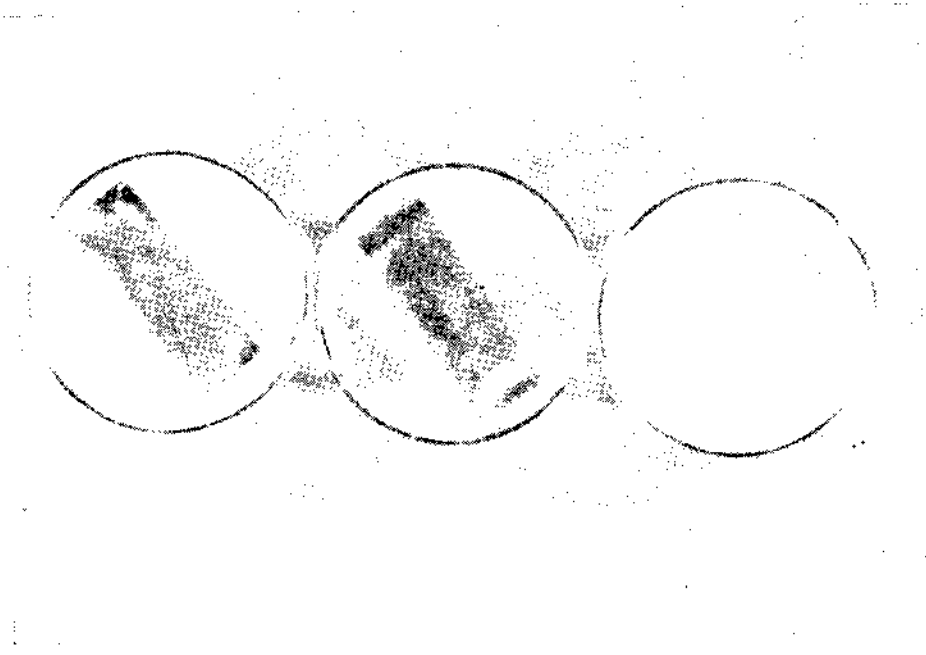
a *b* *c*



a *b* *c*

2

PLATE 2.



a *b* *c*

PLATE 3.

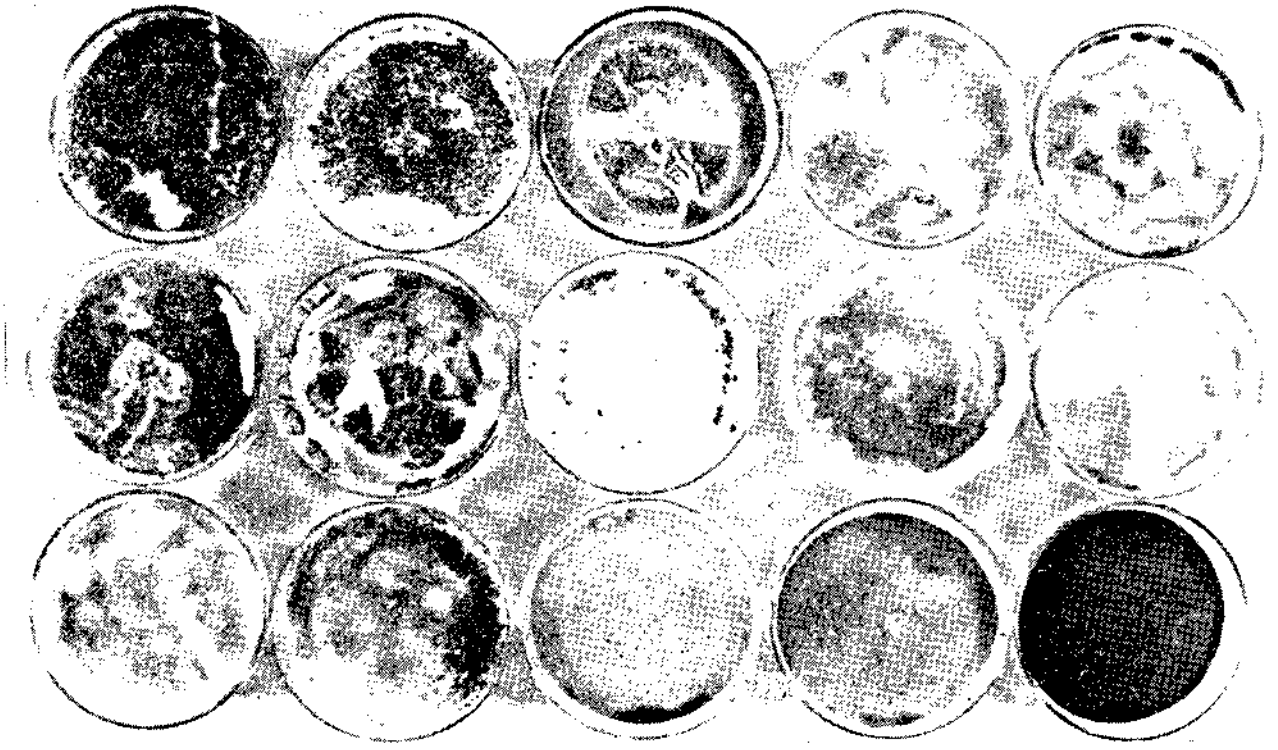


PLATE 4.

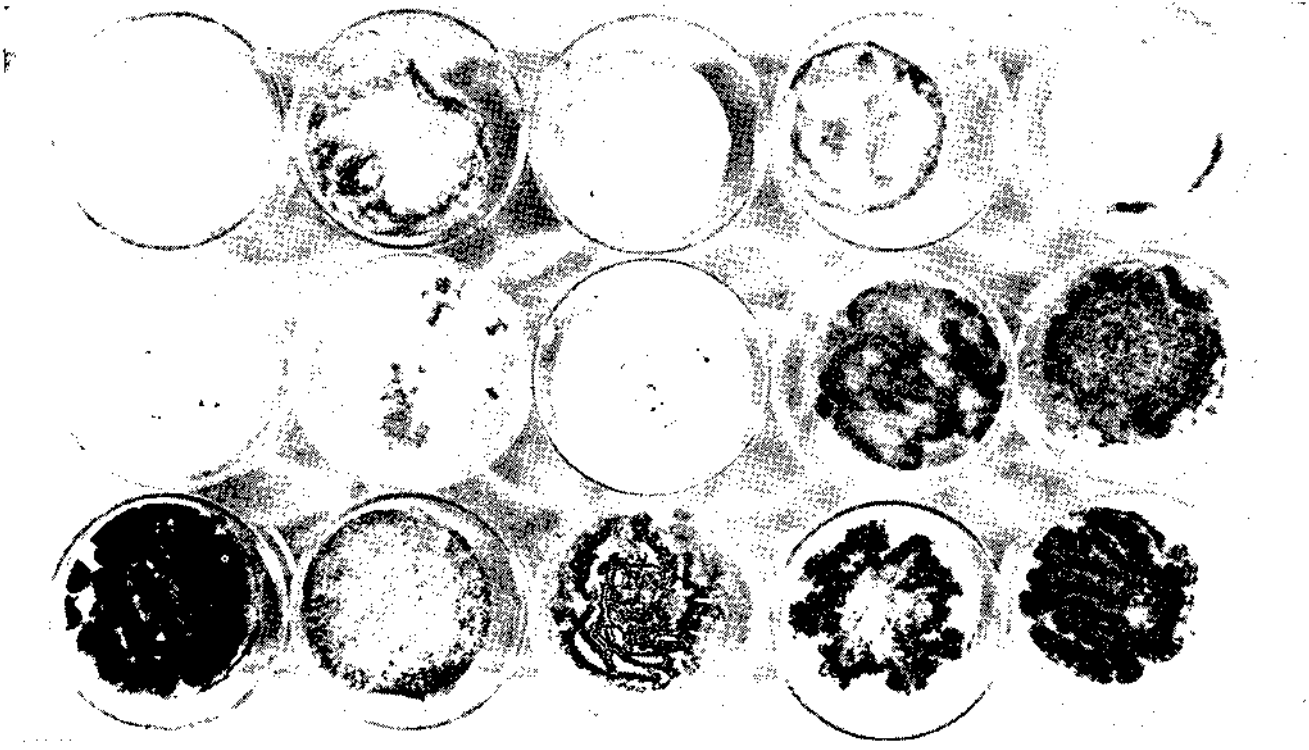


PLATE 5.

EFFECTS OF GAMMA RADIATION ON THE STORAGE PROPERTIES OF CANDIED JACKFRUIT (ARTOCARPUS HETEROPHYLUS LAM.)

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TWO TEXT FIGURES

ABSTRACT

Candied jackfruit (*Artocarpus heterophylus* Lam.) of various moisture levels were prepared using osmotic dehydration and the conventional method of syruping with subsequent oven-drying at varied periods of time. The resulting products were irradiated at 0, 150, 300, and 500 Kr. "Osmotic dried" samples and the oven-dried products with moisture content of 53.8 and 34.1 per cent, respectively, were discarded after less than one month storage due to spoilage and worm infestation. Irradiation of as high as 500 Kr did not improve their keeping quality. Initial microbial counts for samples with low moisture levels (13.9 to 27.1 per cent) were comparatively lower than those with high moisture values (34.1 and 53.8 per cent) and counts for the former samples decreased with increase in irradiation dose treatments. This indicates that there is a critical moisture content (27.1 per cent) to which candied *langka* must be dried if irradiation is to be beneficial in decreasing microbial load. Irradiation appeared to have improved the texture of the candied fruits with moisture levels of 20.5, 18.8, and 13.9 per cent.

INTRODUCTION

Fruit and fruit products have long been important articles of commerce and for many centuries have been a part of man's diet. The application of scientific methods and principles in the fruit product industries has been comparatively recent particularly in the Philippines. Although lately, notable advances have been made in the knowldege of the fundamental scientific principles underlying the processes used in fruit product manufacturing industries, there still remains much to be done on the development and/or improvement of local fruit products. One of such products is the glazed fruit which may either be in the form of cubelets as the glazed fruit mix or as candied segments.

Good quality fruit mix and candied fruits available locally are mostly imported and very expensive. Latest data showed that the Philippine's importation of candied fruits for the year 1970 amounted to P3,385,557.50, a considerable drain in the country's dollar reserve [Private Communication, CBP (1971)]. There are varieties of local fruits that have been demonstrated to be good

materials for the manufacture of candied fruit among which is the jackfruit, locally known as *langka* (*Artocarpus heterophylus* Lam.).

The success in the manufacture of candied fruits has, however, depended much on processing conditions adopted for the specific raw fruit material used. In general, the moisture content of the candied product affects greatly its keeping quality and organoleptic properties. Products with low moisture value (5 per cent) keep longer but are generally unacceptable in view of their tough and leathery texture and unattractive appearance. Those with higher moisture levels (20 per cent and above) are highly acceptable but are susceptible to microbial spoilage. Irradiation may help minimize problems of spoilage encountered in candied fruits of high moisture levels, and consequently preserve their aesthetic qualities.

Irradiation treatment of fruits after harvest have attracted attention all over the world for the control of market diseases, insect infestation, delay of ripening and prevention of sprouting [Sommer and Maxie (1966)]. However, very little research on irradiated dehydrated fruits are available in the literature. According to Dharker and Sreenivasan (1966), semidried bananas of 40-per cent moisture, irradiated with dose level of 0.5 Mrad kept well for at least 3 months, compared with the dehydrated product of 10-per cent moisture content. The latter, besides being susceptible to mold infestation, also possesses poor attributes of color, flavor, retention of nutrients and reconstitutability.

Irradiation appears to hold some promise for the treatment of dry products [Cornwell (1966), Dennison (1967), Golumbic and Davis (1966)]. Current irradiation treatment on dried foodstuffs is directed primarily to the practical objective of controlling insect infestation in order to prevent storage losses and to extend storage life. Since fungi (yeast and mold) are the second most important factor in storage losses and deterioration of dried foodstuffs, like candied fruits, radiation effects on this particular group of microorganisms are also receiving attention. Emphasis is on the reaction of the mold, yeast and insect larvæ when normally occurring in the dried food rather than in their isolated state [Cornwell (1966), Golumbic and Davis (1966)].

Microbiological spoilage in candied fruits is largely associated with bacteria, yeast and molds. The desired degree of microbial effect which could serve as quality control measure, generally determines the radiation dose to be applied.

This paper aims to establish the most suitable irradiation treatment and moisture level that will preserve candied jackfruit at ambient condition at a minimum storage time of 6 months.

MATERIALS AND METHODS

Preparation of candied jackfruit of varied moisture levels.—Jackfruit of eating-ripe quality were cut in wedges and the thick segments were collected, deseeded and trimmed. The prepared segments were divided into two lots. The first lot was subjected to osmotic dehydration process while the other lot was processed using the conventional syruping method of candying fruits. The processes are described as follows:

Osmotic dehydration.—One part by weight of prepared segments were soaked in one part refined sugar overnight. The sugar-soaked segments were then dipped in cold water for a few seconds and drained.

Conventional method.—Prepared segments were soaked in hot 50° Brix syrup and allowed to stand overnight. Syrup concentration was stepped-up daily 5°Brix until concentration reached 70°. The segments were soaked for one week in the 70°Brix syrup, then drained, washed immediately with warm water and dipped momentarily in 5-per cent pectin solution. The segments were divided into six portions and dehydrated in a forced draft oven at 60°C for different periods of time, namely: 0, 1, 2, 3, 4, and 5 hours.

The osmotic-dried and oven-dried segments were packed and sealed in polyethylene bags and representative samples irradiated at room temperature with doses of 0, 150, 300, and 500 Kr using Cobalt 60 facility. Composite experimental samples were tested initially for moisture, microbial load in terms of total plate count (TPC) and yeast and mold count (YMC), and organoleptic properties. The products were stored at ambient condition (Temp. 27-30°C; R.H. 73-80 per cent) and examined on the third and sixth month of storage for moisture, microbial load and organoleptic properties. Incidence of spoilage was likewise observed during these storage periods.

The following methods of analyses were used in the examination of the experimental products: Moisture—AOAC (1965); Total Plate Count and Mold and Yeast Count—Harrigan and McCance (1966).

Organoleptic evaluation in terms of eye appeal, palatability, and texture, was performed by a trained panel of tasters using the

TABLE 1.—*Moisture contents of nonirradiated and irradiated candied before and during 6 months storage at room temperature.*

Dose/storage period	Moisture Content (Per cent)						
0 Kr Initial	53.8*	34.1	27.1	22.0	20.6	18.1	13.9
3rd month	-	-	23.9	22.8	19.9	18.8	16.1
6th month	-	-	23.9	23.9	18.5	18.5	17.0
150 Kr Initial	49.4*	36.2	27.8	23.3	17.3	22.2	15.4
3rd month	-	-	27.2	24.5	18.4	17.9	15.3
6th month	-	-	25.5	21.1	21.7	19.2	19.1
300 Kr Initial	49.1*	34.8	29.0	22.6	21.4	19.0	14.8
3rd month	-	-	27.6	18.8	19.6	19.0	17.3
6th month	-	-	25.6	23.9	15.4	19.3	18.5
500 Kr Initial	50.3*	33.5	24.1	24.1	19.2	20.0	13.4
3rd month	-	-	26.1	20.4	20.9	19.0	17.3
6th month	-	-	26.8	22.6	20.0	20.4	18.4

(—) Not determined. Samples were discarded within the first month due to spoilage and worm infestation.
* Osmotic-dried.

following hedonic rating scale [Peryam and Pilgrim (1957)] for scoring the product: like extremely, 9; like very much, 8; like moderately, 7; like slightly, 6; neither like nor dislike, 5; dislike slightly, 4; dislike moderately, 3; dislike very much, 2; dislike extremely, 1.

RESULTS AND DISCUSSION

In general; irradiation did not considerably affect the initial moisture values of the experimental samples which were found to range from 13.9 to 34.1 per cent for the oven-dried samples and 53.8 per cent for the "osmotic-dried" product (Table 1). During storage, samples with moisture levels of 27.1, 22.0, 20.6, and 18.1 per cent showed also very little change in moisture content while samples with 13.9-per cent moisture exhibited slight increase in moisture values. These changes may have been due to the temperature variations in the room where the samples were stored as well as to the relative humidity of the air in the packaged product. If the relative humidity of the air is correspondingly greater than the water activity (a_w) of the food, the food will take up moisture, or vice-versa, until the food and surrounding air are in equilibrium with respect to moisture [Frazier (1958)]. It is also possible for microorganisms growing in food to change the level of available moisture by release of metabolic water or by fermenting the substrate so as to form free water [Frazier (1958)].

The "osmotic-dried" and oven-dried samples of 53.8- and 34.1-per cent moisture levels, respectively, were discarded due to spoilage. Worm infestation was very much evident in the samples after 5 days' storage. The former sample was totally discarded after 7 days and the latter after 26 days. Their high moisture content and presence of sugar syrup favored worm infestation and microbial spoilage. It is apparent that irradiation up to 500 Kr did not improve the keeping quality of these samples.

Correlation of the total plate count and yeast and mold counts with moisture levels and dose treatments are shown in Figures 1 and 2. The initial total plate count (TPC) and mold and yeast counts of the "osmotic-dried" and oven-dried samples of 53.8- and 34.1-per cent moisture, respectively, were too numerous to count. Microorganisms in these samples survived irradiation dose treatment of as high as 500 Kr. Samples with low initial moisture levels in the range of 13.9 to 27.1 per cent showed a decrease in microbial count with increase in irradiation dose

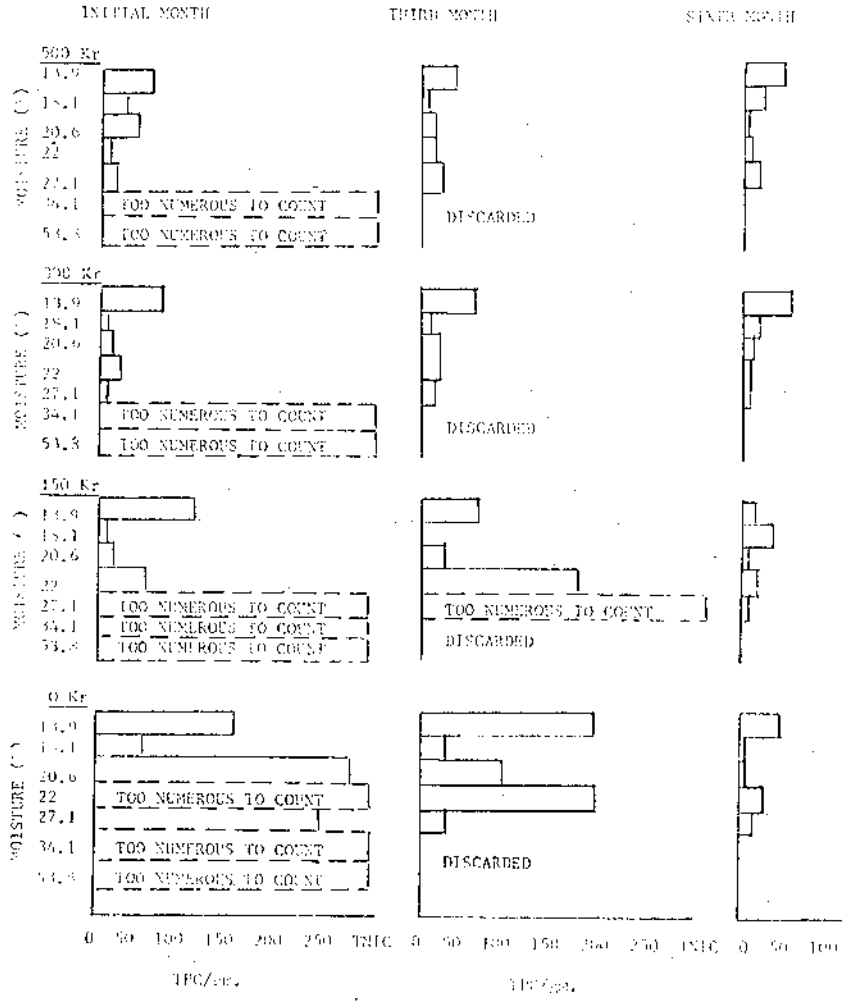

Fig. 1. Total plate count (TPC) of nonirradiated and irradiated candied jackfruit during 6 months of storage at room temperature.

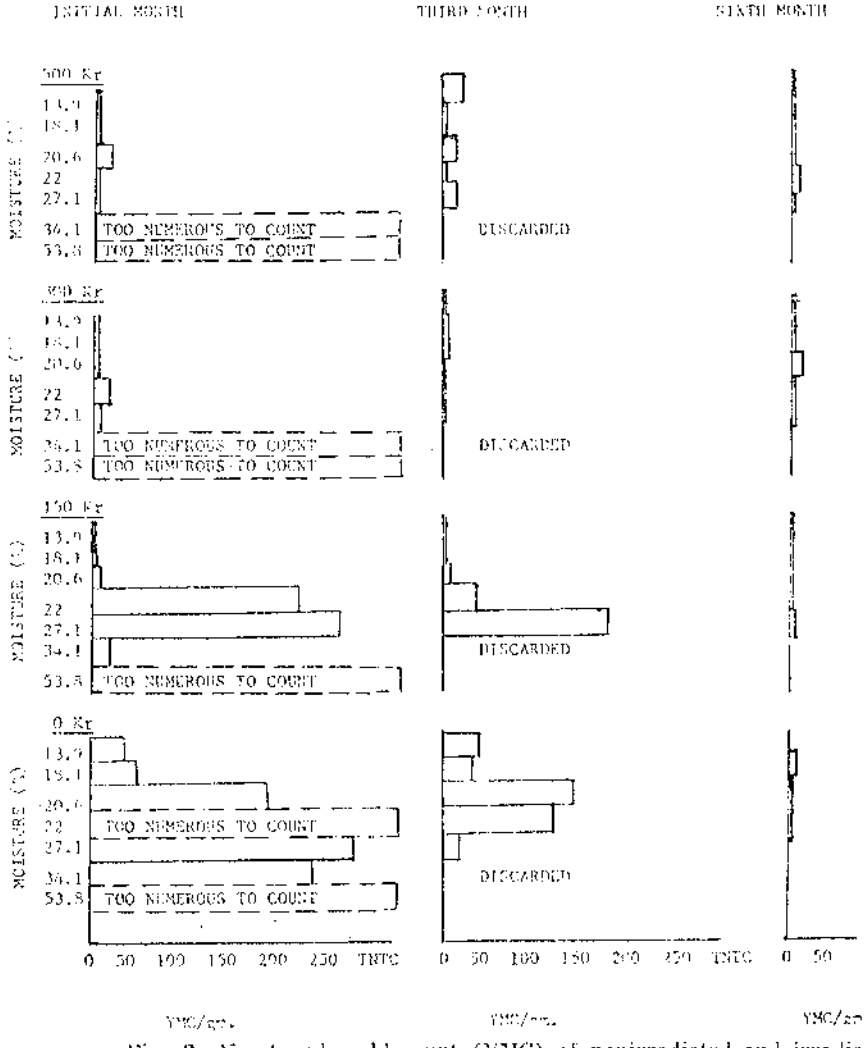

Fig. 2. Yeast and mold count (YMC) of nonirradiated and irradiated candied jackfruit during 6 months storage at room temperature.

treatments. This indicates that there is a critical moisture content (27 per cent) to which candied jackfruit must be dried, if irradiation is to be effective in decreasing microbial load. This moisture content is significantly higher than the levels necessary for good keeping quality in dried fruit ordinarily stored without irradiation.

It has been reported that the direct action of gamma radiation on water molecules in the food enhances free radical formation which indirectly can cause damage or complete destruction of the cells of the organism [Reports Series No. 14, IAEA, Vienna (1970)]. Bacterial destruction is brought about by changes in shape and structure of the cells and alteration of metabolism; reproduction, nutrient requirements which subsequently cause death. The action of radiation on the viable colonies is influenced by the amount of radiation, concentration or numbers of bacteria and chemical composition of the medium [Reports Series No. 14, IAEA, Vienna (1970)]. In the case of candied samples with moisture levels of 30 per cent and above, limited water molecules are attacked by gamma rays, thereby leaving unattacked water molecules which provide an ideal medium for the ultimate growth of surviving spoilage organisms [Sommer and Maxie (1966)]. The results of this experiment are in agreement with this report.

There was a noticeable decrease in the microbial counts of most of the samples during storage which was quite marked on the sixth month storage period. This decrease may be attributed to substances such as acids, alcohols, peroxides, or even antibiotics produced by the microorganisms in foods during storage which are reported to have inhibitory effects on microbial growth [Sommer and Maxie (1966)].

In general, fruits contain a multitude of acids, alcohols, amino acids, organic acids, esters, fat, etc. Unfortunately, little research has been conducted toward determining the effects of low dose radiation on these compounds *in vivo*. The effects are important because of their direct effect on the microbial survival and quality attributes of irradiated fruit and fruit products which may produce delayed changes affecting storage qualities [Maxie and Abdel-Kader (1966)].

The mean acceptability scores received by the various samples before and during the 6-month storage period at room temperature are given in Table 2. Low initial scores in palatability and texture were received by the "osmotic-dried" samples and oven-dried samples with 53.8- and 34.1-per cent moisture content,

TABLE 2.—*Mean acceptability scores of irradiated and nonirradiated candied jackfruit of varied moisture levels during 6 months storage at room temperature.*

Dose/storage period	EYE							APPEARANCE							PALATABILITY							TEXTURE						
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0 Kr																												
Initial	6.67	6.67	6.17	6.33	6.33	6.83	7.50	6.83	6.67	5.83	6.83	7.00	6.83	7.50	6.67	6.50	6.33	6.67	6.50	6.50	6.83							
3rd month	-	-	6.50	6.17	5.83	6.67	7.33	-	-	6.17	6.50	6.33	6.50	7.50	-	-	6.50	6.33	6.17	6.17	6.50							
6th month	-	-	6.33	6.33	6.00	6.17	7.50	-	-	6.50	7.00	7.00	6.17	6.17	-	-	6.33	7.00	7.00	6.33	6.83							
150 Kr																												
Initial	6.17	6.33	6.33	6.33	6.50	6.50	7.67	6.33	5.00	5.17	6.00	7.17	6.33	7.50	6.17	5.17	7.17	6.33	7.67	6.67	7.00							
3rd month	-	-	6.00	6.00	6.17	6.17	6.00	-	-	5.83	6.67	6.33	5.50	7.50	-	-	6.50	6.33	6.17	6.83	7.00							
6th month	-	-	6.00	6.33	6.33	6.33	6.00	-	-	6.17	6.33	6.17	6.33	6.67	-	-	6.00	6.67	6.33	6.00	6.17							
300 Kr																												
Initial	6.67	6.67	7.17	6.33	7.33	7.33	7.67	6.33	6.17	5.17	5.67	7.17	6.33	7.17	6.33	6.67	6.83	5.17	7.33	6.67	6.83							
3rd month	-	-	6.33	6.33	6.83	6.33	6.00	-	-	6.33	7.00	6.17	6.33	7.50	-	-	6.17	6.33	6.50	6.33	7.33							
6th month	-	-	6.17	6.33	6.00	6.33	7.00	-	-	6.17	7.00	6.50	6.50	6.83	-	-	6.50	6.50	6.17	6.33	6.33							
450 Kr																												
Initial	6.83	6.83	6.50	6.67	6.50	7.00	7.00	5.33	6.33	5.00	6.33	6.67	7.33	6.50	6.67	5.17	6.33	6.67	7.17	6.83	7.17							
3rd month	-	-	6.33	6.33	6.17	6.67	7.00	-	-	6.33	6.33	6.00	5.50	7.17	-	-	6.17	6.33	6.00	6.17	6.50							
6th month	-	-	6.67	6.33	6.33	6.00	7.17	-	-	6.00	6.50	6.17	6.33	6.50	-	-	6.33	6.17	6.33	6.50	6.33							

(—) discarded
* osmotic-dried

respectively. These samples were too wet, soft and sticky. This is particularly true with the former which also lacked the sweet flavor, characteristics of candied fruits. In general, higher ratings in the three qualities tested were obtained by the control and irradiated samples with moisture levels of 13.9 to 22.0 per cent as compared with the candied product containing 27.1-per cent moisture. Analysis of variance showed that irradiation at the dose levels used did not affect significantly at 5-per cent level the organoleptic qualities of the experimental products. However, irradiation appeared to have improved the texture of candied fruits with moisture levels of 20.6, 18.1, and 13.9 per cent as evidenced by the higher scores obtained by these samples. Irradiation has been reported to improve texture and rehydration capacities of dried vegetables. These textural changes have been associated with the effects of irradiation related to radiation-induced degradation in the structural polysaccharides of the foodstuffs such as pectin and cellulose [Massey and Bourke (1967)]. Pectin and cellulose which are also naturally occurring in fruits were reported to be degraded by approximately the same dose at which tissue softening could be first demonstrated and progressed with increasing dose in a manner similar to that of softening itself.

In between storage periods, there was no significant difference in texture of the experimental samples at 5-per cent level but showed significant difference in palatability and eye-appeal. Browning was observed on all samples during the first month of storage which affected their acceptability. This undesirable discoloration may be attributed to the effect of irradiation on pectin which was used to coat the finished product since in the preliminary studies conducted previously in which pectin was not utilized, no browning discoloration was observed on the dried candied product. It may be noted at this point, that pectin was utilized in the glazing of the candied fruit in view of its lustrous attractive effect on the finished product which appeared very dull without it.

Pectin, a mixture of several carbohydrates, might have been depolymerized by radiation producing simple sugars which could have reacted with proteins, producing this undesirable color change [Massey and Bourke (1967)]. Monosaccharides, oligosaccharides and polysaccharides respond to radiation by degradation to smaller units i. e., glucose, maltose and other products of irradiation [Maxie and Abdel-Kader (1936)].

SUMMARY

Candied jackfruit (*Artocarpus heterophylus* Lam.) of various moisture levels was prepared using osmotic dehydration and the conventional method of syruping with subsequent oven-drying at varied periods of time. The resulting products were irradiated at 0, 150, 300, and 500 Kr. Irradiation did not considerably affect the initial moisture values of the samples. However, slight changes were noted during the 6-month storage period.

"Osmotic-dried" samples and the oven-dried product with moisture content of 53.8 and 34.1 per cent, respectively, were all discarded after less than 1 month storage due to spoilage and worm infestation. Irradiation to as high as 500 Kr did not improve their keeping quality. Initial microbial counts for samples with low moisture levels (13.9 to 27.1 per cent) were comparatively lower than those with high moisture values (34.1 and 53.6 per cent) and counts for the former samples decreased with increase in irradiation dose treatments. This indicates that there is a critical moisture content (27.1 per cent) to which candied *langka* must be dried if irradiation is to be benificial in decreasing microbial load. This moisture content is significantly higher than the levels necessary for good keeping quality in dried fruits ordinarily stored without irradiation. There was a noticeable decrease in the microbial counts of most of the samples during storage.

Irradiation at the dose levels used did not affect significantly the organoleptic qualities of the experimental products. However, it appeared to have improved the texture of the candied fruits with moisture levels of 20.5, 18.1, and 13.9 per cent. In between storage period; no significant difference in texture of the experimental samples was noted at 5-per cent level but showed significant difference in palatability and eye appeal. Browning was observed in the stored product.

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REFERENCES

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS (1965). 6th Ed. Washington, D.C., 957 pp.

CORNWELL, P. B. (1966). Status of Irradiation control of insects in grain. Food Irradiation Proc. of the Inter. Symp., Karlsruhe, June 6-10, 1966, jointly organized by the IAEA and FAO. IAEA, Vienna, pp. 455-471.

DENNISON, R. A. (1967). Fruit and dry product irradiation processes. Radiation Preservation of Foods. Advances in Chemistry Series 65. Amer. Chem. Soc. Wash., D.C., pp. 152-155.

DHARKAR, S. D., and A. SREENIVASAN (1966). Irradiation of tropical fruits and vegetables. Food Irradiation Proc. of the Inter. Symp., Karlsruhe, June 6-10, 1966, jointly organized by the IAEA and FAO. IAEA, Vienna, pp. 635-649.

FRAZIER, W. C. (1958). Food Microbiology. New York: McGraw-Hill Book Co., Inc., 472 pp.

GOLUMBIC, C., and D. F. DAVIS (1966). Radiation disinfestation of grain and seeds. Food Irradiation Proc. of the Inter. Symp., Karlsruhe, June 6-10, 1966, jointly organized by the IAEA and FAO. IAEA, Vienna, pp. 473-488.

HARRIGAN, W. F., and M. S. MCCANCE (1966). Laboratory Methods in Microbiology. London: Academic Press, 362 pp.

MASSEY, JR. L. M., and J. B. BOURKE (1967). Some Radiation-Induced Changes in Fresh Fruits and Vegetables. Radiation Preservation of Foods. Advances in Chemistry Series 65. Amer. Chem. Soc., Washington, D.C., pp. 1-11.

MAXIE, E. C., and ADEL ABDEL-KADER (1966). Advances in Food Research 15: 105-139.

PERYAM, D. R., and F. P. PILGRIM (1957). Hedonic scale method of measuring food preference. Food Tech. 11: 9-14.

PRIVATE COMMUNICATION. Economic Research Department. Central Bank of the Philippines, Manila, 1971.

SOMMER, N. F., and E. C. MAXIE (1966). Recent Researches on the Irradiation of Fruits and Vegetables. Food Irradiation Proc. of the Inter. Symp., Karlsruhe, June 6-10, 1966, jointly organized by the IAEA and FAO. IAEA, Vienna, pp. 572-587.

TRAINING MANUAL ON FOOD IRRADIATION TECHNOLOGY AND TECHNIGUE. Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture Tech. Reports Series No. 14, IAEA, Vienna (1970) 134 pp.

THE PRODUCTION OF MANGANESE DIOXIDE FROM MANGANESE ORES

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ONE TEXT FIGURE

ABSTRACT

A method is described for the production of manganese dioxide. The process involves the roasting of the ore at 500°C for 2 hours after grinding to the proper size (200 mesh) and a quantity of ammonium sulfate added. The manganese sulfate produced as an intermediate product is leached with water, filtered and the filtrate freed of impurities by a series of pH adjustments. Then ammonium carbonate is added to precipitate manganese carbonate. The manganese carbonate is next converted to manganese nitrate and finally calcined to decompose it to manganese dioxide. A relatively pure product has been successfully produced from low-grade manganese ores.

INTRODUCTION

The Philippines is rich in natural resources, most of which have remained untapped. Although some of these resources have been exploited, others have not been industrially transformed locally into higher-priced processed or semiprocessed products. The result has been that the bulk of these resources like minerals, which are of relevance to this study, logs and copra are exported in their raw or semifinished forms.

One such abundant indigenous natural resource is manganese ore which is of recent mineral development starting only in 1937-1938. Studies show that certain basic chemicals with potentialities for use in various manufacturing industries can be derived or extracted from manganese ores.

Manganese is a gray-white metal resembling iron, but it is harder and very brittle. The compounds of manganese occur in several mineral forms widely distributed throughout the crust of the earth. The most important of these minerals are the oxides, the chief oxides of which are pyrolusite (MnO_2, 63.2-per cent Mn), psilomelane (colloidal form of MnO_2, 45 to 60-per cent Mn), manganite ($Mn_2O_3 . H_2O$, 62.4-per cent Mn), and hausmannite (Mn_3O_4).

The chemical grade MnO_2 finds application in several industries such as in paint, glass, ceramics, as well as dry-cell battery manufacture. In metallurgical industries it is used particularly in the production of ordinary carbon-steels and special-high manganese steels.

In the dry-cell battery production it is used in the form of its oxide in which high-grade pyrolusite (MnO_2) is required for this purpose. The ore for battery manufacture should contain not less than 84-per cent manganese dioxide, but usually in the range of 85 to 90 per cent. The ores with lower manganese dioxide content can be used in some electrical application.

In glassmaking, the ore needed usually contains 85- to 90-per cent manganese dioxide and less than 1-per cent iron. But for high quality glass, a manganese dioxide of over 90 per cent and an iron content less than 0.5 per cent is required.

In the ceramic field, manganese dioxide is used to produce brown, purple and black glazes and enamels and to produce slate-colored tiles and bricks [Sully (1955)].

In the paint and varnish industry, manganese dioxide is used as oil drier [Kirk and Othmer, ed. (1932)].

In medicine, it is occasionally used internally in doses of 3 to 20 grains (0.2 to 1.3 g). Officially, it is used in preparing chlorine water (compound solution of chlorine), a solution used as antiseptic and stimulant [Remington (1917)].

Realizing these various needs for manganese and manganese dioxide in particular, this study was undertaken with the object of first making a survey of the different methods of producing manganese dioxide and finally determining the most efficient and economically feasible method suited to local materials and conditions.

REVIEW OF LITERATURE

Manganese reserves in the Philippines are extensive. Although great quantity had already been mined and shipped abroad still there remains a great amount of reserves. An estimate made by the Bureau of Mines showed that as of January to December 1970, manganese ore reserves stood at 2,779,764 M.T. with an average grade of 22.99-per cent Mn.

The grades of manganese ore deposits in different areas and sometimes even in the same area are variable. But for operating mines, the minimum and maximum grades are 23-per cent and 54-per cent Mn, respectively [Philip. Bur. Mines (1954)].

Manganese ores vary a great deal in composition, particularly in the balance of the manganese and iron contents. Since about 95 per cent of the total manganese mined is used for metallurgical purposes the ores are classified on the basis of manga-

nese content and the type of ferro alloy for the kind of manufacture intended.

1. Manganese ores — contain more than 35-per cent Mn. These are suitable for the manufacture of high or low grade ferro-manganese.

2. Ferruginous manganese ores or spiegel ores — contain 10- to 35-per cent Mn. They are used for the manufacture of spiegeleisen.

3. Manganiferous iron ores — contain 5- to 10-per cent Mn. These are used for the manufacture of manganiferous pig iron.

Manganese ore is mostly found in the form of secondary deposits, the manganese having been dissolved out of crystalline rocks and deposited as the carbonate, oxide, or hydroxide. These secondary deposits are sedimentary or residual and the most common mode of occurrence is in the form of wad, braunite, manganite, pyrolusite, or psilomelane.

The major ores of manganese are the oxides in hydrated or dehydrated forms and to a lesser extent the silicates and carbonates. They are as follows [after Sully (1955)]:

1. Pyrolusite (MnO_2) — Its manganese content when pure is 63.2 per cent
2. Psilomelane ($MnO_2.H_2O$) — 45- to 60 per cent Mn
3. Manganite ($Mn_2O_3.H_2O$) — 62.4-per cent Mn
4. Braunite ($3\ Mn_2O_3.MnSiO_3$) — 62-per cent Mn and 8- to 10-per cent silicate
5. Hausmannite (Mn_3O_4)
6. Rhodochrosite ($MnCO_3$) — 47.8-per cent Mn
7. Rhodonite ($MnSiO_3$) — 42-per cent Mn
8. Benentite (hydrated silicate) — 31-per cent Mn and 5-per cent silicate.

From the qualitative standpoint, Philippine manganese may be classified as follows [after Olayco (1956)]:

Class	Per cent Mn	Per cent SiO_2	Per cent Fe	Per cent Al_2O_3
High-grade	53.58	1.36	1.41	3.70
Marginal-grade	45.14	12.86	0.96	2.79
High-silica	40.81	22.46	4.43	4.19
Sub-marginal	32.64	18.69	11.39	3.35
Low-grade	20.02	14.72	13.55	11.81

Of the above classes of ores, the low-grade ore constituting fairly big deposists in the Philippines poses the most serious problem. Special treatments are necessary and these must be influenced by economic considerations.

Geologists and mining engineers of the Bureau of Mines who have made a survey and study of the deposits (old or new), are of the opinion that with systematic development, these deposits will constitute an important source of manganese now and in the years to come. Deposits which have been newly discovered are located in the provinces of Samar, Catanduanes, Surigao, Capiz, Tarlac, Bulacan, and Oriental Misamis. Although the economic possibilities of these new sources have not yet been fully evaluated, in the course of time the ore reserves in these areas will be known [Philip. Bur. Mines (1952)]. However, the more known deposits are located in Bohol, Camarines Sur, Negros Oriental, Masbate, Palawan, Ilocos Norte, and Zamboanga del Sur [Basco (1962)].

In industrially rich and advanced countries like the U.S.A., the beneficiation and utilization of the low-grade manganese ore is being tackled with considerable degree of enthusiasm. In the Philippines, likewise, considerable interest has already been generated to extensively locate and explore new areas and to improve mining methods. But beneficiation and other metallurgical investigations have yet to be undertaken to dress up low-grade ores to marketable quality.

A survey of literature has shown that manganese dioxide may be produced chemically by various methods like the Nossen Nitric Acid Cycle [Nossen (1951)], Digestion with Hydrochloric Acid [Prasad (1954)], and Roasting with Ammonium Sulfate [Stringham and Summers (1965)].

The Nossen Nitric Acid Cycle developed by E.S. Nossen Laboratories [Nossen (1951)] and the Digestion with Hydrochloric Acid by Krishna N.S. Prasad (1942) are processes which both involve at the start, the reduction of the ore in a reducing atmosphere to manganese oxide. Exploratory experiments were conducted to test the adaptability of these methods to local conditions but due to lack of facilities these two methods were discontinued and instead studies were centered on a third process (roasting with ammonium sulfate to produce a manganese salt) developed by Stringham and Summers (1965). This method involves the roasting of the manganese ore with a quantity of ammonium sulfate at a certain temperature and time to produce

manganese sulfate ($MnSO_4$). The manganese sulfate produced is converted to manganese carbonate ($MnCO_3$), then to manganese nitrate [$Mn(NO_3)_2$] and finally decomposed to manganese dioxide [MnO_2].

EXPERIMENTAL PROCEDURE

The samples of manganese ore used in our experiments were tetragonal crystals, heavy and compact, hard and sooty with iron-black metallic color.

The sources of raw materials (manganese ore) used in our study are Zambales Base Metals and Acoje Mining Co. with the following composition:

	First batch Zambales Base Metals	Second batch Acoje Mining Company
	Per cent	*Per cent*
Mn	45.24	57.18
SiO_2	6.61	2.65
Fe	2.05	1.81
P	3.25	2.75
Al_2O_3	0.09	0.05

Based on the above results of analysis, the samples may be classified as natural ores of psilomelane, $MnO_2.H_2O$. It can not be considered a silicate since silica content is lower than that of Braunite ($_3MnO_2.MnSiO_3$) with 8 to 10 per cent. It cannot be classified as a carbonate because upon treatment with HCl, no effervescence was observed.

The manganese ore was crushed and ground to 200 mesh. Fifty grams of the ground material were mixed with a definite amount of ammonium sulfate. The mixture was thoroughly blended and roasted in a retort at a definite temperature and roasting time. After cooling the roast (the calcine) it was weighed and leached with water to form a 15-per cent solution of manganese sulfate ($MnSO_4$), then allowed to stand for a few hours and finally filtered. The residue was dried and assayed for the amount of manganese that might still be present.

It was expected that by the roasting process all of the Mn would be converted to $MnSO_4$. At the start our main concern was to determine the best roasting time and temperature, and the right ratio of ore and ammonium sulfate that would extract practically all the manganese from the ore thus rendering it soluble with hardly a trace of manganese in the residue.

The solution or filtrate ($MnSO_4$) which should now have a pH of 2.3 was freed of its impurities of iron, silica and phosphorus by a series of pH adjustments.

The above solution with a pH of 2.3 was adjusted to pH 4.5 with ammonium hydroxide to precipitate ferric hydroxide, then to pH 5.4 with calcium hydroxide to precipitate phosphorus as calcium phosphate, then to pH 7 with ammonium hydroxide, allowed to settle, then filtered. Finally, sufficient ammonium carbonate was added to raise the pH to 8.5 to precipitate manganese carbonate which was filtered and then washed. Manganese carbonate was then calcined at 400°C for 2 hours. The residue was mixed with an equal weight of nitric acid and heated at 150° to 200°C to remove the nitrogen oxides. This was finally calcined to decompose the manganese nitrate to manganese dioxide.

A schematic diagram of the above procedure is shown in Figure 1.

Several sets of experiments were conducted to determine the effects of varying conditions like amounts of ammonium sulfate (ratio of Mn: $(NH_4)_2SO_4$, 1:1 to 1:7); temperature (500°C to 900°C); and duration of roasting (1 to 4 hours) period; and temperature (200°C to 300°C) and duration (2 to 3 hours) of calcination of the manganese nitrate [$Mn(NO_3)_2$] to the final product manganese dioxide (MnO_2). In order to evaluate the variations in the process, analyses of the materials in the process were made.

RESULTS AND DISCUSSION

The five tables show the conditions under which the experiments were conducted. The results presented in Tables 1 to 4 are averages of seven trials each using the first batch of manganese ores from Zambales. Results in Table 5 made use of manganese ores from Acoje Mining Company in Baguio.

It is evident from Table 1 that as roasting time was increased there was a corresponding decrease in the amount of manganese left in the residue.

The results presented in Table 2 show that at a lower temperature there was a corresponding decrease of the pH and the amount of manganese in the residue. Therefore at very high temperatures (900° to 700°) the extraction procedure is likely to produce poor results.

It may be observed from Table 3 that at the ratio of 1:5 for Mn ore: $(NH_4)_2SO_4$, the assay of the manganese in the residue was almost nil although the pH still remained at 3.2.

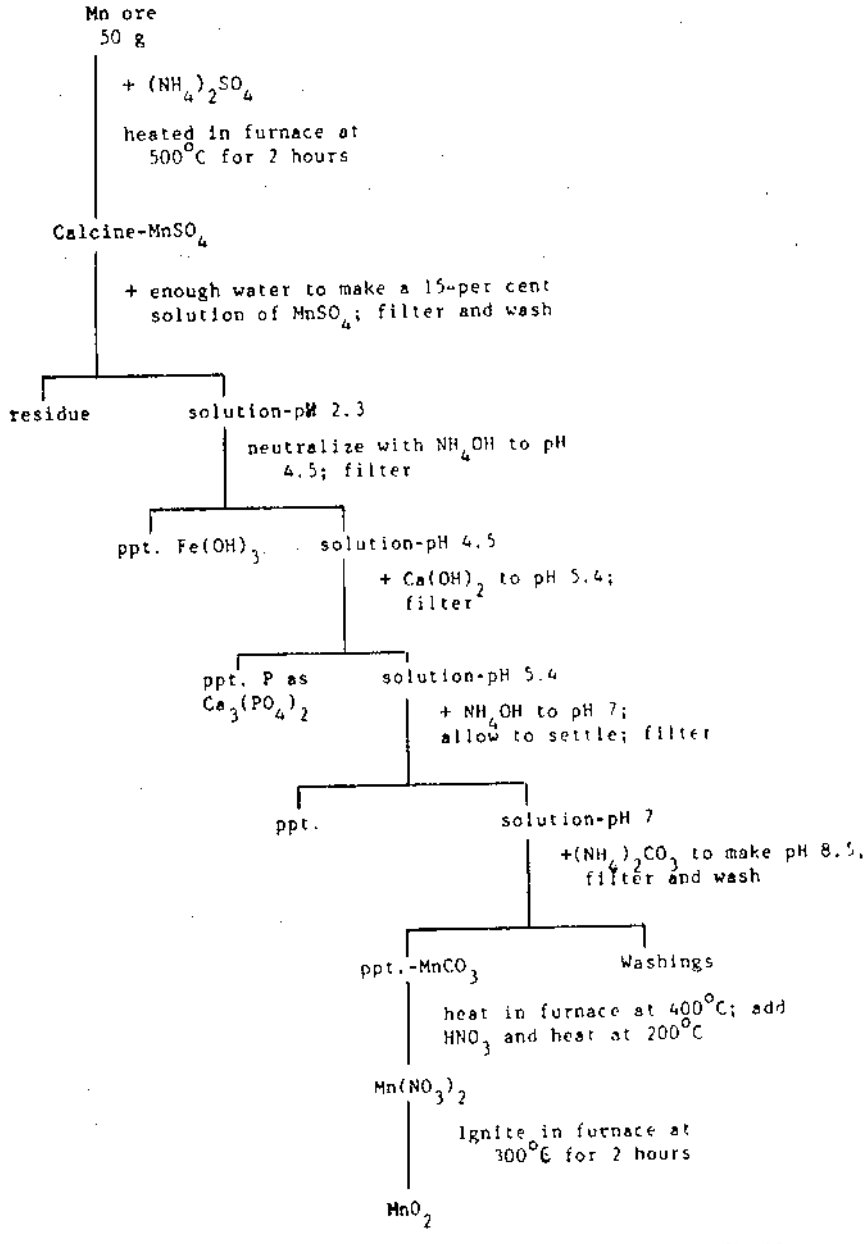

FIG. 1. Flow diagram for the production of manganese dioxide.

From the results gathered in Tables 2 and 3 it was deduced that better extraction of the manganese from the ore may be attained at still lower temperatures and higher ratio of the ammonium sulfate to the ore.

To test this assumption another roasting experiment was conducted at 500°C but at 1:4 ratio of ore and ammonium sulfate.

TABLE 1.—*Effect of varying roasting time on degree of extraction.*

Roasting temperature: 600°C Ratio of ore to ammonium sulfate: 1:1	
Roasting time hr	Assay of residue Per cent Mn
1	14.52
2	11.88
3	8.56

TABLE 2.—*Effect of varying roasting temperature on the degree of extraction.*

Roasting time 3 hours Ratio of ore to ammonium sulfate: 1:1		
Roasting temperature °C	pH	Assay of residue Per cent Mn
900	6.4	34.00
800	6.2	15.50
700	6.0	10.20
600	5.0	8.56

TABLE 3.—*Effect of varying ratio of manganese ore to ammonium sulfate on the degree of extraction at 600°C roasting temperature.*

Roasting temperature Roasting time		600°C 3 hours
Ratio of ore: $(NH_4)_2SO_4$	pH	Assay of residue Per cent Mn
1:2	4.0	8.03
1:2.5	3.6	5.03
1:3	3.4	1.39
1:3.5	3.7	0.72
1:4	3.7	0.42
1:4.5	3.6	0.41
1:5	3.2	0.13

TABLE 4.—*Effect of varying ratio of manganese ore to ammonium sulfate on the degree of extraction at 500°C roasting temperature.*

Roasting temperature Roasting time		500°C 3 hours
Ratio of ore: $(NH_4)_2SO_4$	pH	Assay of residue Per cent Mn
1:3	2.8	0.60
1:3.5	2.8	0.25
1:4	2.8	0.15
1:4.5	2.8	0.13
1:5	2.8	0.07
1:6	2.9	0.20

TABLE 5.—*Summary of results obtained using the optimum conditions established.*

Roasting temperature	:	500°C
Roasting time	:	2 hours
Manganese to ammonium sulfate ratio	:	1:13
Calcination temperature of manganese nitrate to manganese dioxide	:	300°C
Calcination time	:	2 hours

Trials	Weight of Sample	Amount of Mn in sample	MnO_2	Yield	Recovery of Mn
	g	*g*	*Per cent*	*Per cent*	*Per cent*
1	100	57.18	95.61	82.02	86.80
2	100	57.18	98.70	79.81	83.85
3	100	57.18	98.51	76.00	82.90

Results showed a manganese content of 0.15 per cent in the residue and a lower pH of 2.8 after leaching which indicated a more complete conversion of the manganese in the ore to soluble manganese sulfate.

Using 500°C as the roasting temperature and 3 hours as the roasting time, more sets of experiments using varying amounts of $(NH_4)_2SO_4$ were conducted. Results are shown in Table 4. It can be seen that at the ratios of 1:3 to 1:5 the pH remained constant at 2.8 and at the ratio of 1:5 the assay of the manganese in the residue was almost nil. At the ratio of 1:6 the per cent

manganese in the residue increased and the pH which is 2.9 is still far from the expected pH of 2.3.

So more experiments were conducted, this time to find out how a shorter roasting time and a higher ratio of the ammonium sulfate to the Mn ore would improve the degree of extraction and give the desired results.

The results showed that at 500°C, 2-hour roasting time and a 1:6 or 1:6.5 manganese to ammonium sulfate ratio the pH was almost 2.3 (pH 2.2 to 2.4) and the Mn in the residue almost nil (0.1752 to 0.0080 per cent).

Based on these results using the ratio 1:6 (Mn ore: $(NH_4)_2SO_4$), the ratio of the equivalent Mn content (of the ore) to the ammonium sulfate would be 1:13 as explained in the computations below:

100 g ..... 600 g
Mn ore $(NH_4)_2SO_4$
(46.1 per cent)

$$1{:}6 \qquad \frac{600}{46.1} = 13$$

From the above ratio, constant 13 was derived, which if multiplied by grams manganese in the ore would give the number of grams of $(NH_4)_2SO_4$ that would be roasted with it, thus $46.1 \times 13 = 600$ g $(NH_4)_2SO_4$. So a manganese ore sample containing 46.1-per cent Mn would be using 600 g $(NH_4)_2SO_4$ per 100 g of ore.

The optimum conditions now established are:

Temperature of roasting ..... 500°C
Roasting time ..... 2 hours
Ratio of Mn (in ore) to $(NH_4)_2SO_4$ 1:13

After obtaining these conditions, experiments were continued to separate and precipitate impurities. This was followed by the precipitation of manganese carbonate ($MnCO_3$), its conversion to manganese nitrate [$Mn(NO_3)_2$], and its final calcination to manganese dioxide (MnO_2).

Experiments were conducted again this time to compare the effect of temperature (200°C and 300°C) and time (2 to 3 hours) of calcination of Mn $(NO_3)_2$ on the purity of the product. Results in Table 5 showed the high percentage purity of manganese dioxide produced after calcination at 300°C for 2 hours.

SUMMARY

A relatively pure product of manganese dioxide has been successfully prepared from local manganese ores. Optimum con-

ditions for recovering as much as 87 per cent of the manganese in the ores have been established. Essentially, the method consists of roasting the ground ore with a required amount of ammonium sulfate at 500°C for a period of 2 hours. The calcine, after cooling, is leached with water and the filtrate freed of its impurities of iron, phosphorus, and silica by a series of pH adjustments. Then sufficient ammonium carbonate is added to precipitate manganese carbonate. Manganese carbonate is then converted to manganese nitrate and finally decomposed to manganese dioxide by roasting at 300°C for 2 hours.

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REFERENCES

Basco, D. (1962). The manganese bearing limestone deposits in Anda Peninsula, Bohol and their economic significance. Philip. Soc. Mining Metall. Geol. Eng. Newsletter, Manila **14** (1): 7-9.

Kirk, R. E., and D. F. Othmer, ed. (1932). Encyclopedia of Chemical Technology **8**: 718, 747.

Hamilton, L. F., and S. G. Simpson (1946). Determination of oxidizing power of pyrolusite. Talbot's Quantitative Chemical Analysis. 11th edition, New York: Macmillan Co., pp. 237-241.

Nossen, E. S. (1951). Manganese concentration from low grade domestic ore. Ind. Eng. Chem. **43**: 1690-1700.

Olayco, I. (1956). The Philippine manganese problem. Philip. Soc. Mining Metall. Geol. Eng. Newsletter, Manila **8** (2): 30.

Philippine Bureau of Mines (1952). Manganese in the Philippines. Bur. Mines Inform. Circ. (10): 1-28.

Philippine Bureau of Mines (1954). Mineral resources of the Philippines. Bu. Mines Inform. Circ. (19): 66-74.

Prasad, K. N. S. (1954). Preparation of manganese dioxide from low-grade manganese ores. Jour. Indian Inst. Sci. **36** (1): 19-22.

Remington, J. P. (1917). Mangani Dioxidum Praecipitatum. U.S.P. The Practice of Pharmacy. 6th edition. Philadelphia and London: J.B. Lippincott Co., pp. 855-856.

Stringham, W. S., and G. N. Summers (1965). Beneficiating manganese ores. U.S. Patent No. 2,724,645, November 22, 1965. *In* Chem. Abst. (1966) **64**: 15424c.

Sully, A. H. (1955). The occurrence and ores of manganese. Manganese. London: Butterworth Scientific Publications, pp. 3-10.

Zambo, J., and B. Dezco (1964). Production of manganese dioxide from manganese carbonate. Fenujs Kuta Int. Keglemen, Hungary **7**: 381-93. *In* Chem. Abst. (1966) **64**: 15424e.

STEROLS FROM SARGASSUM POLYCERATIUM MONTAGNE AND S. CONFUSUM AGARDH

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FIVE TEXT FIGURES

ABSTRACT

Fucosterol (I) was isolated from the unsaponifiable portion of the fat of *Sargassum polyceratium* and *Sargassum confusum*, and identified on the basis of its infrared and mass spectra.

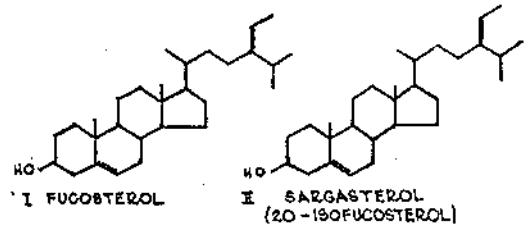
Marine and fresh-water algæ are widely distributed in the Philippines. They are utilized as food and as source of agar, alginic acid and other industrial products [Sulit *et al* (1952)]. A systematic chemical survey of this group has been carried out to investigate the algæ in detail. Work in this field has included a study of polysaccharides, pigments, fatty acids, and sterols.

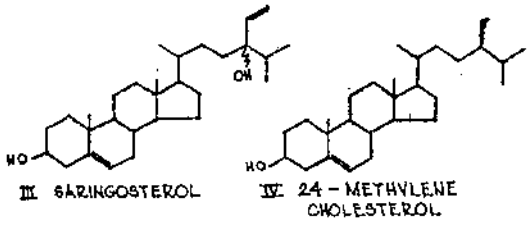
The first intensive study of sterols from algæ was conducted by Heilbron *et al* (1934). The occurrence of nine different sterols in algæ was reported by Miller (1962) in his review on fats and steroids. The brown algæ have been found to be most consistent in their sterol content with fucosterol (I) occurring in all the species investigated [Heilbron *et al* (1934), Ito *et al* (1956), Motzfeldt (1970). Shirahama (1942 and 1936), Tsuda *et al* (1958a-1958b)].

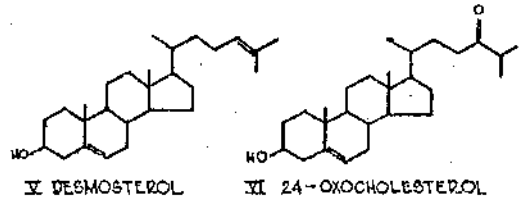
Aside from fucosterol, *Sargassum ringgoldianum* [Tsuda *et al* (1958a)] and *Eisenia bicylis* [Tsuda *et al* (1958b)] yielded sargasterol (II). More recently saringosterol [Ikekawa *et al* 1966)] (III) was obtained with fucosterol from *S. ringgoldianum* and *Dictyopteris divaricata*.

Ikekawa *et al* (1968) determined the composition of nine phaeophytes by gas chromatography and mass spectrometry. Fucosterol was the main component with smaller amounts of cholesterol, 24-methylene cholesterol (IV) and saringosterol. Patterson (1968) also reported the presence of the above mentioned sterols

and, tentatively of desmosterol (V) in *Laminaria foeroensis* and *L. digitata*. Aside from fucosterol, Motzfeldt (1970) found small amounts of 24-oxocholesterol (VI) in *Pelvetia canaliculata*.







The known sterols (published data) of brown algæ are summarized in the table below.

Order	Family	Genus and species	Sterols	Reference
Dictyotales	Dictyotaceæ	*Dictyopteris divaricata*	Fucosterol and saringosterol	Ikekawa *et al* (1968)
		Padina arborescens	Fucosterol	Ito *et al* (1956)
Chordariales	Chordariaceæ	*Heterochordaria abietina*	Fucosterol	do
Laminariales	Laminariaceæ	*Laminaria angustata*	Fucosterol	do
		L. digitata	Fucosterol, 24-methylene cholesterol and	

			tentatively desmosterol	Patterson (1968)
		L. japonica	Fucosterol	Ito *et al* (1956)
		L. ochotensis	Pelvesterol	Shirahama (1942)
		Costaria costata	Fucosterol	Tsuda *et al* (1958a)
		Eisenia bicyclis	Fucosterol and sargasterol	Tsuda *et al* (1958b)
		Undaria pinnatifida	Fucosterol	Ito *et al* (1956)
		Alaria crassifolia	Fucosterol	Shirahama (1942)
Fucales	Fucaceæ	*Fucus evanescens*	Fucosterol	Tsuda *et al* (1958a)
		F. vesiculosus	Fucosterol	Heilbron *et al* (1934)
		Pelvetia canaliculata	Fucosterol and 24-oxocholesterol	Motzfeldt (1970)
		P. wrightii	Fucosterol	Tsuda *et al* (1958a)
	Sargassaceæ	*Cystophyllum hakodatense*	Fucosterol	do
		Hizikia fusiformis	Pelvesterol	Shirahama (1936)
		Sargassum ringgoldianum	Fucosterol and sargasterol	Tsuda *et al* (1958a)
			Fucosterol and saringosterol	Ikekawa *et al* (1966)

Among the algæ in the Philippine waters, species of the genus *Sargassum* have been the most studied [Anonymous (1946), de Leon *et al* (1963), and Sulit *et al* (1952)]. These are the brown algæ which are of economic importance as source of alginic acid [Sulit *et al* (1952)] and utilized as fertilizer for tubers [Anonymous (1946)]. They were also included by Montilla and Blanco (1952) in their list of edible and medicinal seaweeds. De Leon *et al* (1963) determined moisture, ash, nitrogen, crude fat, crude fiber, iodine, mannitol and alginic acid contents of *Sargassum* and other algæ.

The present work is an investigation of the sterol content of *Sargassum polyceratium* and *S. confusum*. Sterols which closely resembled fucosterol, the typical sterol of brown algæ, were isolated from them. It is hoped that this study will open the field for a greater utilization of Philippine algæ.

EXPERIMENTAL

Preparation of algæ for extraction.—*Sargassum polyceratium* and *S. confusum* collected on October, 1969, from exposed shores of Moron, Bataan were sorted, washed and sun-dried. The dried algæ were cut into small pieces and ground in an electric mill.

Extraction of nonsaponifiable lipids.—A given weight of finely powdered dry algæ were extracted exhaustively with benzene in Soxhlet extractors. The extract was concentrated under partial vacuum. The greenish-black oil obtained was saponified by refluxing with 4-per cent methanolic KOH at 40° for 4 hours. The methanol was distilled off under partial vacuum. The concentrate was diluted with 50-ml water and extracted with benzene several times. The extract was dried over anhydrous Na_2SO_4, and the solvent was distilled off leaving a brownish-orange residue representing the nonsaponifiable lipids. The yield of crude algal extracts is given in the table below.

Yield of crude algal extracts

Samples	Weight of sample (g)	Crude benzene extract		Nonsaponifiable lipids	
		Wt (g)	Per cent	Wt (g)	Per cent
S. polyceratium	334.0	4.15	1.21	1.01	0.30
S. confusum	350.0	3.36	0.96	0.46	0.13

Isolation and identification of sterols.—The nonsaponifiable lipids were dissolved in hot methanol under reflux. The methanolic solution was cooled. Crystals which deposited were separated from the mother liquor by decantation. Recrystallization from methanol gave needlelike colorless crystals:

S. polyceratium : 61.5 mg
S. confusum : 60.8 mg

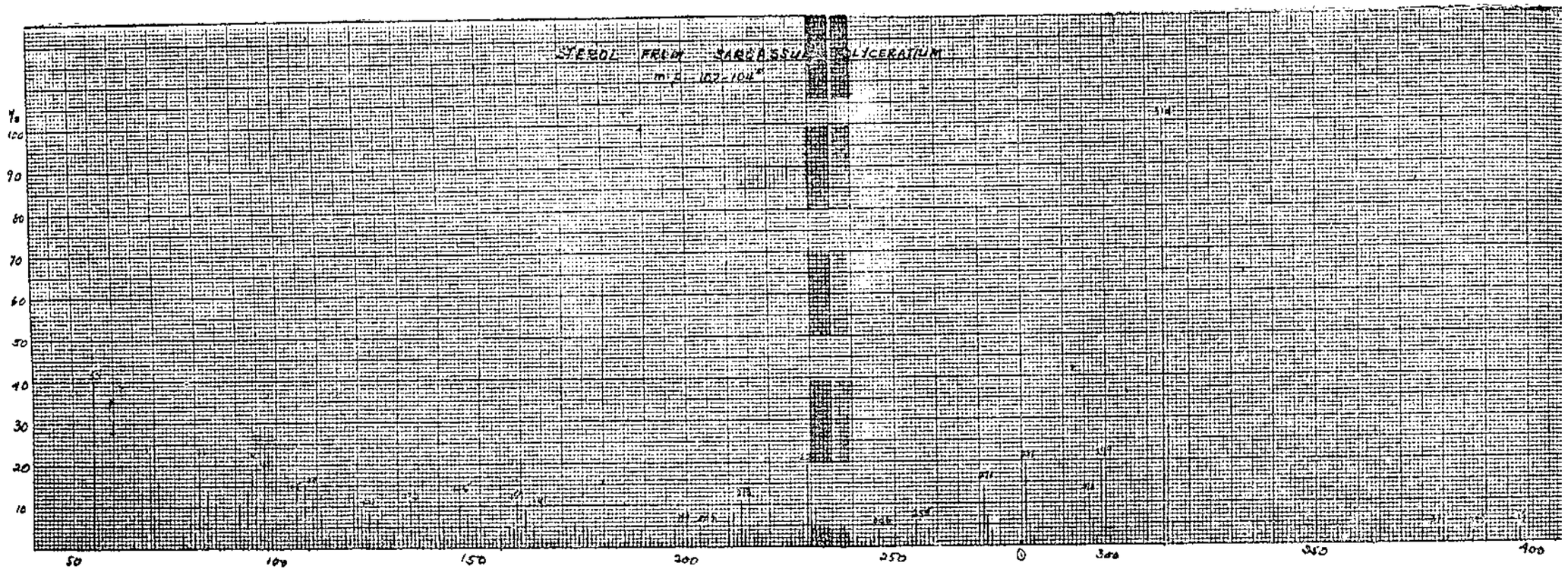
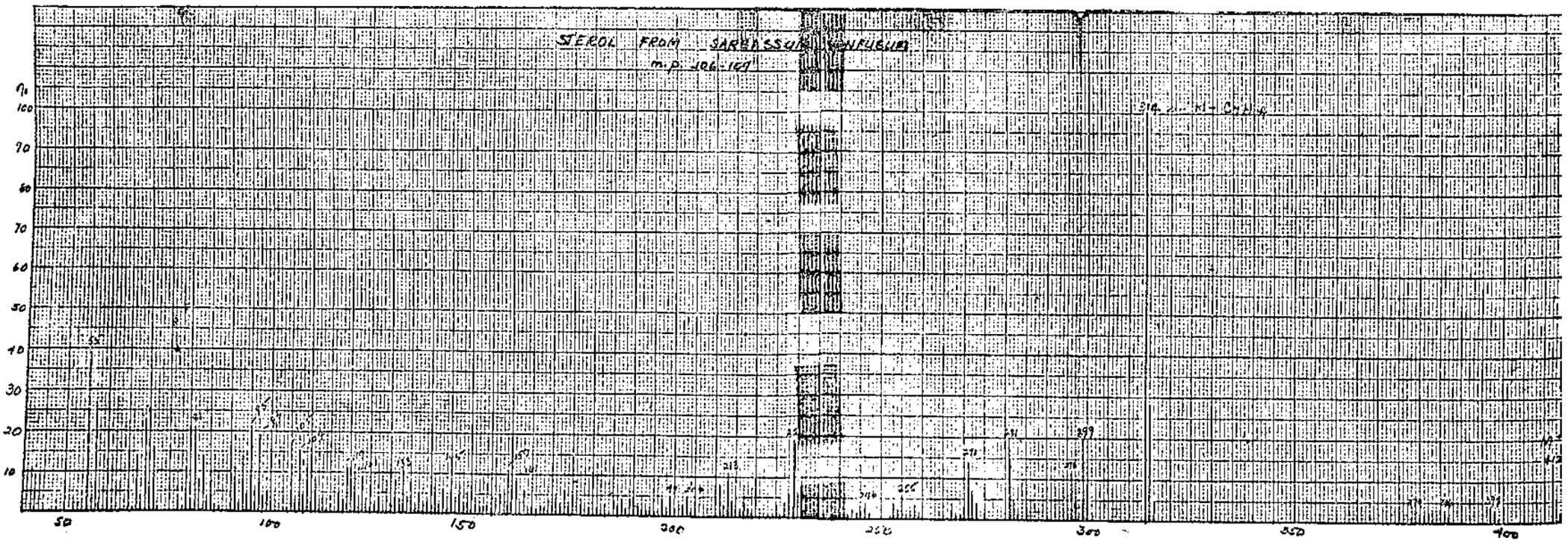


FIG. 4. Mass spectrum of sterol isolated from *Sargassum polyceratium*.



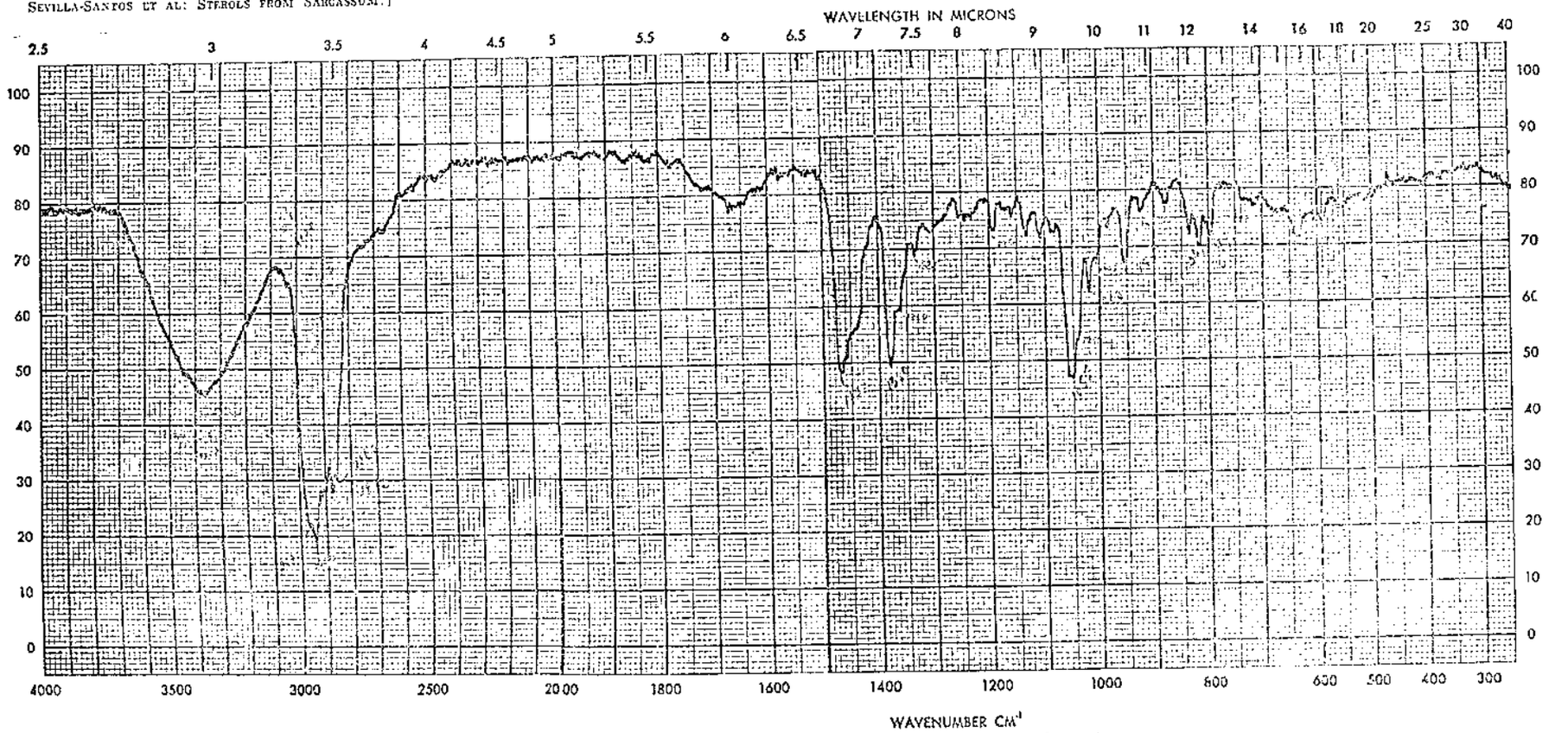


FIG. 2. IR spectrum of sterol isolated from *Sargassum polyceratium*.

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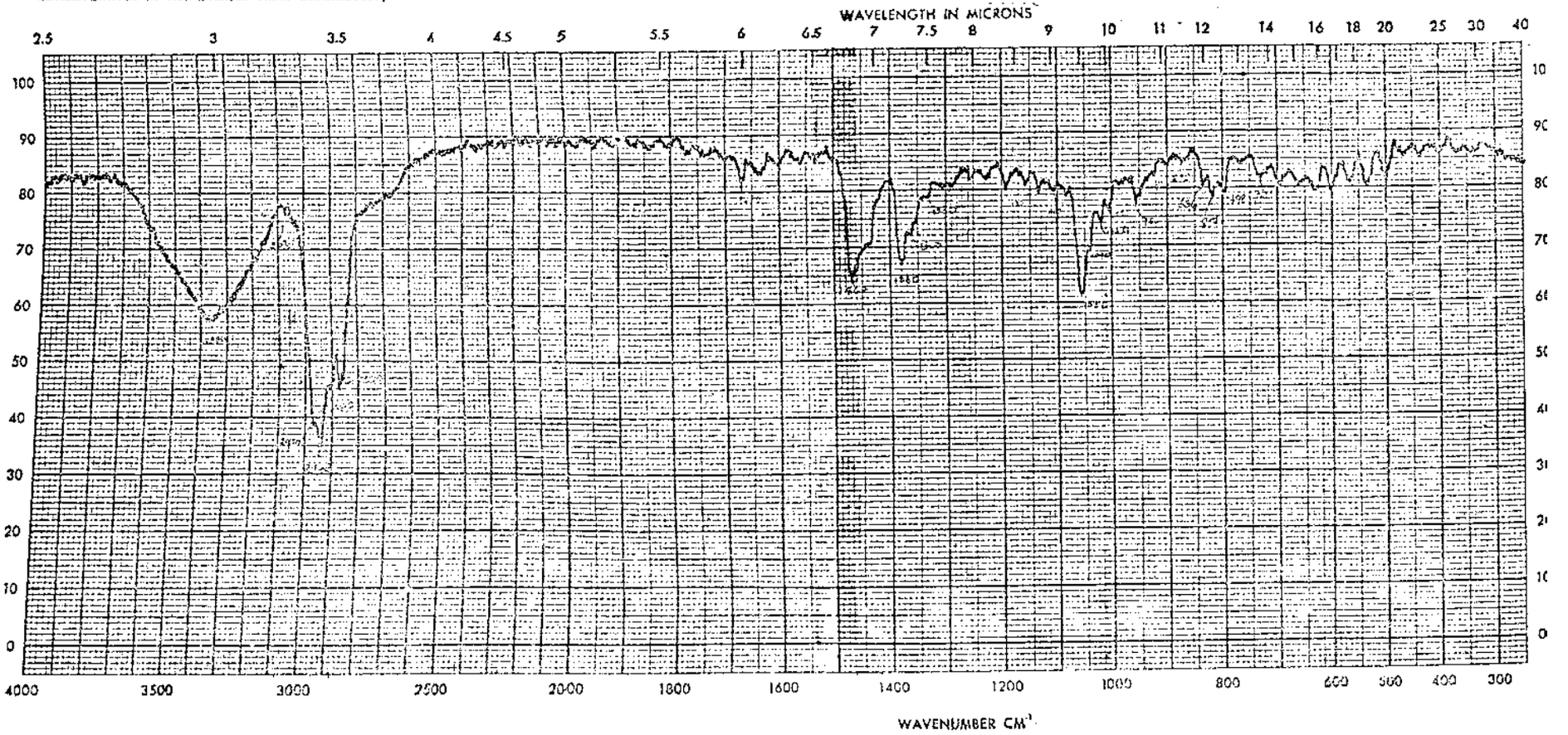

FIG. 3. IR spectrum of sterol isolated from *Sargassum confusum*.

Liebermann-Burchard test gave a typical slow-reacting blue-green color of $\triangle^5$ steriods which suggested the presence of sterols.

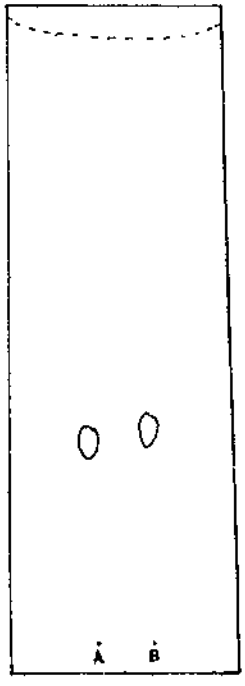

FIG. 1. TLC of chloroform-methanol solutions of: A. Sterol from *S. polyceratium*; B. Sterol *S. confusum*.

The R_f values on silica gel G of the crystals obtained from *S. polyceratium* and *S. confusum* in the solvent system ether/hexane/acetic acid (70:30:0.5) and visualized in iodine vapors were 0.327 and 0.348; respectively (Fig. 1).

The sterols were recrystallized to constant melting point from methanol. Melting points determined in a Kofler type apparatus were 102 to 104° and 106 to 109° for *S. polyceratium* and *S. confusum*, respectively.

Infrared spectra were determined as solid suspensions of the samples in KBr discs using a Beckmann Spectrophotometer. The infrared spectra of the sterols of *S. polyceratium* (Fig. 2) and *S. confusum* (Fig. 3) resembled that of fucosterol [Gibbons *et al* (1968)] with the following peaks:

Sterols from *S. polyceratium*	Sterols from *S. confusum*	Fucosterol [Gibbons *et al* (1968)]	Interpretation
798cm^{-1}	798cm^{-1}	798cm^{-1}	5,6 double
836	836	842	bond
816	816	822	Out-of-plane bending of H atom on a tri-substituted ethylene such as the 24, 28 double bond.
1055	1055	1050	O-H bending and C-O stretching vibration of
3370	3370	3400	hydroxyl group. Intramolecularly H-bonded OH.

Mass spectrometry of both algal sterols gave spectra (Figs. 4-5) similar to fucosterol [Bergman *et al* (1965)] with a parent ion at m/e 412 and the following fragments:

	Sterols from *S. polyceratium*	Sterols from *S. confusum*	Fucosterol [Gibbons *et al* (1968)]	Interpretation
m/e	397	397	397	M^+CH_3
	379	379	379	$M^+(CH_3+H_2O)$
	314	314	314	M^+part of the side chain C_7H_{14}
	299	299	299	$M^+C_7H_{14}+CH_3$
	296	296	296	$M^+(C_7H_{14}+H_2O)$
	281	281	281	$M^+(C_7H_{14}+CH_3+H_2O)$
	271	271	271	M^+(side chain+2H)

SUMMARY

Sterols were isolated from the nonsaponifiable portion of the lipids of *Sargassum polyceratium* and *S. confusum* by fractional crystallization. The infrared and mass spectra of both algal-sterols resembled those of fucosterol, the typical sterol of brown algæ.

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REFERENCES

ANONYMOUS (1946). Cultivation and utilization of some commercial seaweeds of the Philippines. Dept. of Agriculture and Commerce. Food Prod. Ser. Leaflet No. 7: 1-7.

BERGMAN, J., B. LINDGREN, and C. SVAHN (1965). Triterpenes in birchwood. Acta Chem. Scand. 19: 1661-6.

DE LEON, A., N. EUFEMIO, and M. PINEDA (1963). Chemical composition of some Philippine algæ. Philip. Jour. Sci. 92: 77-87.

GIBBONS, G., L. GOAD, and T. GOODWIN (1968). The identification of 28-isofucosterol in the marine green algæ Enteromorpha intestinalis and Ulva lactuca. Phytochem. 7: 983-8.

HEILBRON, I. M., R. F. PHIPERS, and H. R. WRIGHT (1934). The chemistry of the algæ, Part I. The algal sterol-fucosterol. Jour. Chem. Soc., pp. 1572-6.

IKEKAWA, N., K. TSUDA, and N. MORISAKI (1966). Saringosterol: A new sterol from brown algæ. Chem. Ind. 27: 1179-80.

IKEKAWA, N., N. MORISAKI, K. TSUDA, and T. YOSHIDA (1968). Sterol composition in some green and brown algæ. Steroids 12: 41-8. Thru Microbiol. Abs. 4 (4): A1858.

ITO, S., T. TAMURA, and T. MATSUMOTO (1956). Fucosterol of some brown algæ. Nippon Daigaku Kogaku Kenkyusho Sho. **13**: 99-103. Thru CA **53**: 13276 d.

KANEDA, T. (1952). The oils and sterols of algæ. II. The structure of a sterol of Sargassum ringgoldianum. Bull. Japan Soc. Sci. Fish. **17**: (819): 20-24. Thru CA **48**: 13828 b.

MILLER, J. D. A. (1962). Fats and steroids. In Lewin's Physiology and Biochemistry of Algæ, 357-70 pp.

MONTILLA, J., and G. BLANCO (1952). Marine products of minor commercial importance. Philip. Fisher., 111-24 pp.

MOTZFELDT, A. M. (1970). Isolation of 24-oxocholesterol from marine brown algæ Pelvetia canaliculata. Acta Chem. Scand. **24** (5): 1846-7. Thru CA **74**: 978 c.

PATTERSON, G. W. (1968). Sterols of Laminaria. Comp. Biochem. Physio. **24** (2): 501-5. Thru CA **68**: 66349 f.

SHIRAHAMA, K. (1935). Unsaponifiable matter of algæ fats. I. Sterols. Jour. Agr. Chem. Soc. Japan II: 980-84. Thru CA **30**: 1416.

SHIRAHAMA, K. (1942). Unsaponifiable matter and phospatides in marine algæ fats. Jour. Faculty Agr. Hokkaido Imp. Univ. **49** Part I. Thru CA **42**: 8265 c.

SHIRAHAMA, K. (1936). Unsaponifiable matter of the algæ fats. II. Pelvesterol from Hizikia fusiformis. Jour. Agr. Chem. Soc. Japan. **12**: 521-2. Thru CA **30**: 6786.

SULIT, J. I., O. B. NAVARRO, and R. C. SAN JUAN (1952). Chemical studies and utilization of some Philippine seaweeds. Proc. Indo-Pacific Fisher. Coun., 165-170 pp.

TSUDA, K., R. HAYATSU, Y. KISHIDA, and S. AKAGI (1958a). Sterol studies. VI. Studies on the constitution of sargasterol. Jour. Am. Chem. Soc. **80**: 921-5.

TSUDA, K., S. AKAGI, R. KISHIDA, R. HAYATSU, and K. SAKAI (1958b). Steroid studies, IX. Sterols from ocean algæ. Chem. Pharm. Bull. **6**: 724-7. Thru CA **54**: 17459 f.

TRIMETHYLAMINE AND VOLATILE REDUCING SUBSTANCES IN FRIGATE MACKEREL (AUXIS THAZARD LACEPEDE)*

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SIX TEXT FIGURES

ABSTRACT

Two chemical indices of keeping quality of fish were investigated. The VRS (volatile reducing substance) and TMA (Trimethylamine) content of fish were determined quantitatively and correlated with organoleptic and bacteriological tests. The TMA and VRS values increased with increase in temperature of storage and gradual deteriorative changes in the fish could be detected using the chemical tests.

Gutted samples were found to accumulate more bacteria than the round samples. Round mackerel yielded lower TMA and VRS contents compared to the gutted samples. Likewise, the head spoiled more readily than the tail and mid, the latter having the least amount of TMA and VRS. The temperature of storage has been found to be directly related to the formation of VRS and TMA in fish. Relatively lower TMA and VRS obtained at lower, than at higher temperature.

INTRODUCTION

Preservation and shelflife studies of fishery products as well as the determination of the quality of wet fish require the use of a reliable yardstick of keeping quality.[1] Chemical studies on the quality assessment of Philippine fishes are quite limited. Temperate fishes like cod (Castell, 1959), haddock (Farber; 1953); salmon and halibut (Conway, 1962), and tuna (Yamagata, 1971) have been studied by workers using objective methods.

Soon after death, fish attains a condition known as *rigor mortis* which is regarded as a state of excellent quality (Partmann, 1965). This state is followed by a more complicated change known as *autolysis*, which is enzymatic and biochemical in nature. At this stage, enzymes present in the cells catalyze the partial hydrolysis of the tissue proteins leading to softening and changes in flavor and odor. More advanced deteriorative changes due to increase in bacteria and proteolytic enzymes lead to the formation of odoriferous substances and eventually; to the final stages of *putrefaction* (Amlacher, 1961).

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[1] The term "quality" shall be restricted to indicate freshness, a composite of desirable characteristics in fish, which is gradually lost as degradation sets in.

The shelflife of processed products and the acceptability of wet fish have been based mainly on subjective or organolopetic examination. The limitations of sensory evaluation have long been recognized (Farber, 1956). The use of bacteriological tests has been found to be of limited value if isolated from other factors causing fish spoilage (Jacobs, 1951).

The fact that spoiling fish is composed of highly complex substances (Amlacher, 1961) implies the need for more sensitive methods of measuring the degree of fish spoilage. Investigators on food spoilage have devised chemical means by which deteriorative changes could be accurately and quantitatively measured throughout their course.

Atkinson (1965) noted that certain chemical changes in spoiling fish appear to run parallel with the changes in odor, texture, appearance and flavor. Attempts have been devised to estimate the degree of spoilage by the use of analytical procedures for specific chemical substances such as indole, H_2S, histamine, or a group of similarly related end-products of decomposition such as volatile nitrogenous compounds, volatile fatty acids or carbonyl compounds (Farber, 1965). These chemical methods, however, have been found to be of limited use due to the influence of several factors such as type of fish, variation in structure and chemical composition and bacterial flora in fish during spoilage.

Farber (1965) claims that of all the chemical tests studied the VRS determination approaches the sensory judgment of odor and spoilage in a consistent and reliable manner; and is widely applicable to all species of fish and fisheries products so far tested. Local studies by Atilano (1965) and Romero (1964) show that the VRS test offers a good means of evaluating the quality of raw and processed fish since it is most closely correlated with the organoleptic tests.

The VRS (volatile reducing substances) is a composite of all the odoriferous compounds which are chemically reactive to an alkaline solution of $KMnO_4$ at room temperature, and the quantitative determination makes use of a principle which is similar to the olfactory process. A current of air containing the volatile reducing substances is drawn into the alkaline solution of permanganate, which is used as the "chemical nose" (Farber, 1965).

Another chemical means of assessing the quality of fish especially of the marine type, is by the detection of TMA or tri-

methylamine (Conway, 1962). Trimethylamine is produced in spoiling fish muscle by the bacterial reduction of TMA O (trimethylamine oxide). The chemical method employed is capable of measuring semimicroquantities of TMA, such that it can give useful information on the early onset of spoilage. Like the VRS, the TMA production largely depends on certain factors such as type of fish (Shewan, 1956), temperature (Frazier, 1965), and the conditions of storage and processing treatment (Farber and Lerke, 1956).

The present work deals mainly with the determination of the VRS and TMA content of *Auxis thazard* (frigate mackerel) locally known as "tulingan." A comparison between the TMA and VRS content in fish at varying states of quality and temperature and storage condition or processing treatment, is made in simultaneous correlation with organoleptic and bacteriological tests. Preliminary work on the objective assessment of market quality of fish sold to ordinary consumers is also included.

MATERIALS AND METHODS

Sampling.—Newly captured mackerel fish samples taken from Dalahican Beach, Lucena City, were packed and kept in sufficient ice during transport. Fish samples were gutted, others were kept whole or round and for another series of tests, the round and gutted samples were cut into three parts: head, mid and tail. The samples were kept at varying temperatures after the desired preliminary treatment. The TMA and VRS contents as well as the bacterial count of each were determined periodically.

Standard solutions and reagents.—The following reagents and solutions, the preparation and standardization of which were done according to standard quantitative procedures (Hamilton, 1964), were used in the experiment:

1. 0.02N $KMnO_4$ (potassium permanganate) in 1N NaOH (sodium hydroxide)
2. Sodium oxalate, A.R. ($Na_2C_2O_4$)
3. 0.025N $Na_2S_2O_3$ (sodium thiosulfate) in 0.2-per cent sodium borate
4. 6N, N/50 sulfuric acid (H_2SO_4)
5. 1-per cent starch solution
6. 20-per cent KI (potassium iodide) in 0.1-per cent Na_2CO_3 (sodium carbonate)
7. 10-per cent H_3BO_3 (boric acid) in 0.033-per cent bromocresol green and 0.066-per cent methyl red solution

Procedure for the quantitative study of trimethylamine (TMA) in mackerel.—The procedure used was patterned after Conway's microdiffusion method (Conway, 1962). One ml of boric acid was accurately measured and pipetted into the inside chamber of a Conway unit and in the outside chamber, 1 ml of the muscle filtrate was poured and mixed with the same amount of saturated potassium carbonate (K_2CO_3) solution. The Conway unit was covered immediately with a lid moistened with tragacanth gum which provided an effective seal.

After mixing the sample by rotating the unit gently, it was incubated for 120 minutes at 30°F and the contents of the inside chamber titrated with 0.015N H_2SO_4 solution, up to the end point which was indicated by a change in color (from light green to faint red). An automatic semimicroburette was used for accurate determination of the volume of acid used.

The muscle filtrate used was prepared by grinding 10 g of fish in a mortar with a little amount of sand glass. Fifteen ml of 10-per cent trichloracetic acid (TCA) was added and the mixture was allowed to stand for 10 minutes, then filtered. The TMA is expressed in milligrams per 100 g of sample.

The quantitative study of volatile reducing substances (VRS) in mackerel.—The VRS determination employed was patterned after Lang, Farber, and Yerman (1956). The ground fish flesh was wrapped in a double layer of cheese cloth or gauze, placed in the cylinder of the squeezing apparatus on top of a perforated steel disk and pressure was applied to the piston by the use of the Carver hydraulic press. The press juice was collected from the spout at the base of the cylinder.

Aeration which lasted for a definite period was provided using the Farber apparatus. Five ml of press juice was pipetted into the test tube portion of the aerating flask (Flask A) and aerated for exactly 40 minutes. Air containing the volatile substances from the sample was then simultaneously sucked into the modified iodine reaction flask (Flask B) containing a known volume standard solution of 0.02N $KMnO_4$, by means of the Dyna pump. Immediately after the aeration process the unreacted $KMnO_4$ was analyzed indirectly by titration with 0.0245N $Na_2S_2O_3.5H_2O$, using a semimicro burette. Before titration, 6 ml of 6N H_2SO_4, 3 ml of 20-per cent KI in 1-per cent Na_2CO_3 and 8 drops of 1-per cent starch solution which served as the internal indicator, were added to the partially reduced $KMnO_4$.

The difference between the test and the control titer is directly proportional to the amount of reduction of $KMnO_4$, which is expressed in microequivalents of reduction per 5 ml of press juice.

The microbiological study of mackerel.—Bacterial counts of the fish samples (Kreuzer, 1965) were determined using three dilutions: 1:10; 1:100; 1:1,000 (fish homogenate; physiological salt solution). Microorganisms from each dilution were planted into each petri dish containing the solidifying nutrient agar (consisting of bacto-beef extract, peptone, and agar) and incubated for 3 days at 36°F. Bacterial counts were made using the Quebec counter with a tally counter.

The organoleptic examination of mackerel.—Fish samples were organoleptically examined in conjunction with chemical and microbiological tests, based on sensory evaluation of odor, texture, and appearance. The appearance of the eyes, gills, scales, and the discoloration of the flesh especially the belly portion were observed. The texture of the flesh was tested according to its firmness and softness and the ease of separation from the backbone. Changes in the odor, whether seaweedy, ammoniacal, or putrid were noted.

DISCUSSION OF RESULTS

The results obtained from the quantitative study of TMA and VRS in round and gutted fish samples at varying degrees of temperature and days of storage are presented in correlation with organoleptic and bacteriological tests (Tables 1 - 2C).

Results of preliminary tests shown in Fig. 1 indicate that the TMA and VRS values of the samples daily examined increase as spoilage develops. The temperature was maintained between 19 to 20°C since, due to this relatively high temperature, the spoilage as organoleptically tested would develop at a faster rate and hence shorten the period for preliminary tests. Chemical analyses of TMA and VRS content were done daily until the sample reached its last stages of putrefaction. A gradual although small increase in TMA and VRS values obtained was observed for each of the consecutive days of sampling. Daily organoleptic tests, however, did not reveal distinct changes in the quality of the fish. The increase in TMA and VRS content indicate that chemical changes in the fish are slowly taking place. The VRS content ranged from 18.62 to 25.97, while the TMA varied from 3.29 to 7.52. It substantiates the findings of some workers that the VRS

TABLE 1.—*Changes in round and gutted mackerel (Auxis thazard L.) showing variations in TMA and VRS values and log bacterial number during spoilage.*

Days stored	Round	Gutted	V R S		Temp. (°F.)	T M A		Log. bacterial nos.	
			Round	Gutted		Round	Gutted	Round	Gutted
1	Still fresh; flesh firm; odor is freshly seaweedy.	Good; flesh is soft, showing premutolysis; odor seaweedy.	10,780	11,270	10.0	1.882	2.833	4.8903	5.1900
3	Good; flesh soft; odor is seaweedy.	Acceptable; flesh soft; odor slightly seaweedy.	12,010	13,230	15.0	3.293	3.761	5.3768	5.3993
5	Acceptable; flesh softer; odor slightly sweet.	Acceptable; flesh soft; odor slightly ammoniacal.	11,230	17,680	8.0	4.235	4.704	5.5503	5.5750
7	Acceptable; flesh softer; odor strongly sweet.	Not acceptable; flesh very soft; odor slightly ammoniacal.	16,660	21,070	9.9	4.704	5.175	6.1323	6.1432
10	Acceptable; although near its borderline; flesh softer; odor slightly ammoniacal.	Not acceptable; flesh softer; odor ammoniacal.	18,620	22,790	11.0	5.174	5.645	6.4112	6.4359
15	Not acceptable; flesh very soft and flabby; odor strongly ammoniacal.	Not acceptable; flesh very soft, odor ammoniacal.	27,690	26,950	20.0	5.645	5.914	7.1895	7.1934
18	Not acceptable; flesh very soft; retains finger impressions; odor strongly ammoniacal.	Not acceptable; flesh very soft, easily torn from back.	29,640	33,080	20.9	6.586	7.056	7.8732	7.9095
20	Not acceptable; flesh very soft; retains finger impression, odor putrid.	Not acceptable; flesh easily torn from backbone; odor strongly putrid.	38,220	44,690	25.5	7.526	8.469	8.1272	8.1540

TABLE 2a.—*Changes in round and gutted mackerel (Auxis thazard L.) showing variations in VRS content of head, mid and tail portions during storage.*

Days stored	Organoleptic judgment		V R S					
	Round	Gutted	Round			Gutted		
			Head	Mid	Tail	Head	Mid	Tail
1	Fresh fish; flesh firm and elastic; indentation restored; odor freshly seaweed.	Still fresh; flesh firm; odor seaweedy.	9,340	7,540	8,575	10,340	9,555	10,250
5	Still fresh; flesh firm; odor slightly seaweedy.	Considered good; flesh softer; odor seaweedy.	10,780	9,310	9,800	12,405	11,760	12,250
7	Considered good; flesh slightly soft; odor seaweedy.	Considered good; flesh softer due to autolytic changes; odor slightly sweet.	12,560	11,760	12,610	14,210	12,660	13,720
10	Still acceptable; although near its borderline; flesh very soft showing its postmortolysis; odor ammoniacal.	Not acceptable; flesh very soft; odor ammoniacal.	19,560	18,860	19,360	21,560	19,660	21,070
15	Not acceptable; flesh very soft and flabby; retains the finger impressions; odor strongly ammoniacal.	Definitely not acceptable; flesh very soft; easily torn from the back.	27,440	26,250	26,950	34,750	33,610	34,300

TABLE 2b.—*Changes in round and gutted mackerel (Auxis thazard L.) showing variations in TMA content of head, mid and tail portions during spoilage.*

Days stored	Organoleptic judgement		TMA					
			Round			Gutted		
	Round	Gutted	Head	Mid	Tail	Head	Mid	Tail
1	Fresh fish; flesh firm and elastic; indicating postrigor; odor freshly seaweedy.	Still fresh; flesh firm; odor seaweedy.	2.352	0.9408	1.411	2.882	1.882	2.352
5	Still fresh; flesh first odor slightly seaweedy.	Considered good; flesh softer; odor seaweedy.	3.291	1.882	2.352	3.760	2.352	2.833
7	Considered good; flesh slightly soft; odor seaweedy.	Considered good; flesh softer due to autolytic changes; odor slightly sweet.	4.234	2.352	3.760	4.704	2.823	4.234
10	Still acceptable; although near its borderline; flesh very soft showing its post-autolysis; odor ammoniacal.	Not acceptable; flesh very soft; odor ammoniacal.	5.174	3.290	4.234	6.115	4.704	5.174
15	Not acceptable; flesh very soft and flabby; retains the finger impressions; odor strongly ammoniacal.	Definitely not acceptable; flesh very soft; easily torn from the back.	6.115	4.704	5.645	6.586	5.174	6.115

TABLE 2c.—*Logaritim of bacterial plate counts of head, mid and tail portions from round and gutted mackerel (Auxis thazard L.) against storage time and temperature.*

Days stored	Temperature (°C)	Logarithm bacterial numbers					
		Round			Gutted		
		Head	Mid	Tail	Head	Mid	Tail
1	-9.5	4.58659	4.36959	4.48273	4.60756	4.41414	4.50803
5	-8.0	4.59076	4.43483	4.51746	4.63028	4.52440	4.58670
7	10.0	5.77495	5.07773	5.6914	5.79092	5.27416	5.27715
10	15.2	6.24822	6.12516	6.20032	6.27761	6.26340	6.27554
15	20.0	6.60217	6.57933	6.59461	6.70424	6.69723	6.70389

content reaches significant amounts even before TMA values start to rise, using the same sample.

It has been common practice to eviscerate the fish prior to storage, with the generally accepted observation that spoilage develops more slowly in gutted than in round samples. Several authors (Castell *et al*, 1956, Farber and Lerke, 1956) claim that

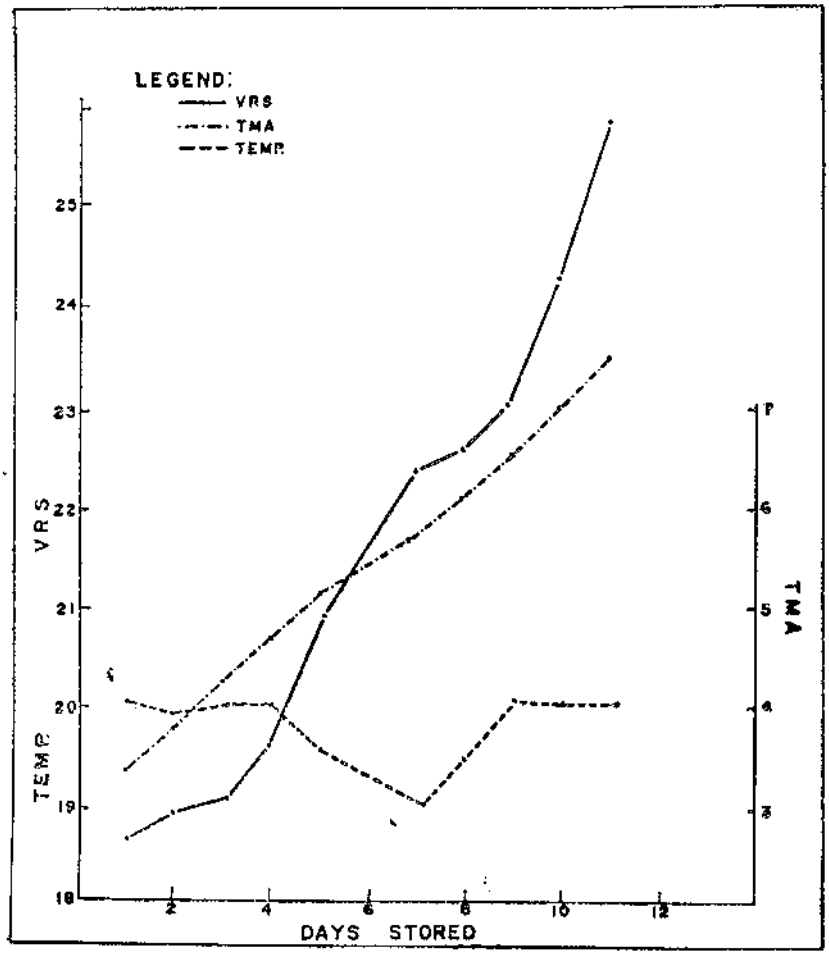


Fig. 1. Preliminary daily tests in round mackerel (*Auxis thazard* Lacepede showing increase in VRS and TMA values with increasing temperature and period of storage.

there is no significant basis for the above procedure, as shown by objective assessment. Proctor, Nickerson, and Goldblith (1950)

also noted that iced, whole, noneviscerated fish do not spoil as quickly as eviscerated or gutted fish similarly refrigerated.

In order to be able to contribute to the findings already made by other workers, similar studies were made, using mackerel samples. Since chemical assessment is objective, the changes in the quality of fish whether round or gutted would be detected without difficulty. It is important to note, however, that the storage temperature must be sufficiently low so that digestive enzymes present in the noneviscerated fish will remain inactive.

Two sets of samples, one round and the other gutted (the viscera and gills removed) were used in the subsequent experiments. Results shown in Table 1 indicate that no appreciable increase in TMA and VRS values occurred in both round and gutted samples between the 1st and 3rd days of storage with slight changes in temperature (10 to 15°F). However, during the first day of storage, the samples which were organoleptically considered as undergoing the later stages of rigor mortis, gave VRS values equal to 10.78 meq. in round samples and 11.27 meq. per 5 ml of press juice, in the gutted samples. The TMA values of round and gutted samples were equal to 1.88 and 2.83 meq., respectively. Consistently higher TMA and VRS values were observed in the gutted rather than the round samples from the first to the tenth day (Fig. 2a). On the 15th day of storage, however, the round samples exhibited slightly higher VRS content. The relatively high temperature (20°F) might have stimulated enzymatic decomposition in the round samples induced by digestive enzymes in the viscera. However, in the 18th to 20th day of storage, gutted samples again gave higher TMA and VRS values probably due to faster increase in the bacterial flora of the gutted fish. It was also noted by Jacobs (1951) that gutted fish exhibited more rapid bacterial decomposition. The bacterial counts of the gutted samples were consistently higher than in the round samples from the first to the 20th day of storage. The evisceration of the fish which caused the breaking up of tissues might have accelerated the entry of microorganisms.

No significant conclusions can be drawn due to lack of sufficient data on other species of fish. However, results using chemical analysis show that mackerel samples can be kept round with no appreciable effect on the keeping quality of the fish. The gutting of the fish to delay the onset of spoilage may not be considered a critical factor as long as the maintenance of low temperature to inhibit bacterial growth and enzymatic activity is carefully observed.

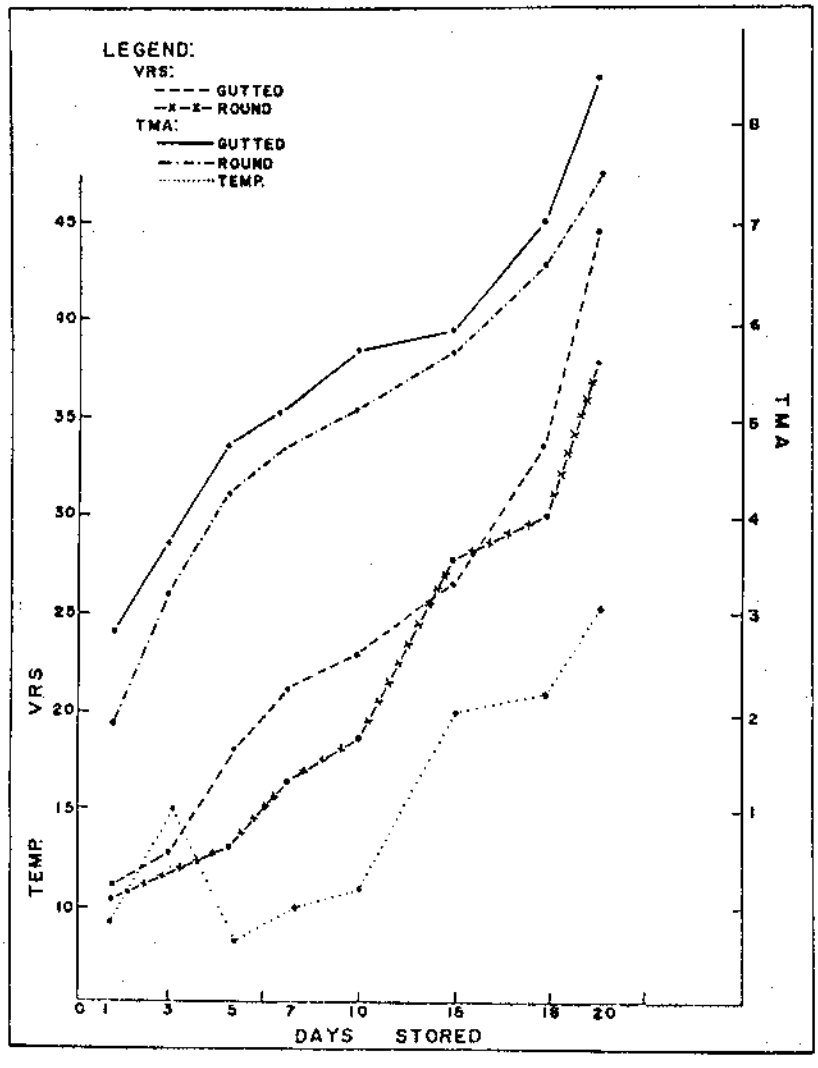


Fig. 2a. Changes in round and gutted mackerel (*Auxis thazard* Lacepede) showing variation of TMA and VRS with temperature and storage time.

Based on the organoleptic judgment (Table 1), the gutted fish were considered unacceptable on the 7th day of storage while the round samples were still nearing definite spoilage and reached its borderline only on the 8th day. After storage periods ranging from 10 to 18 days at an average temperature of 17°F, thousands of bacteria developed rapidly on both samples which

on the other hand, were organoleptically graded as spoiled or deteriorated. A rise in bacterial number accompanied the increase in TMA and VRS values and the difference between the round and gutted fish is also reflected in the bacterial count although at a less appreciable degree (Fig. 2b).

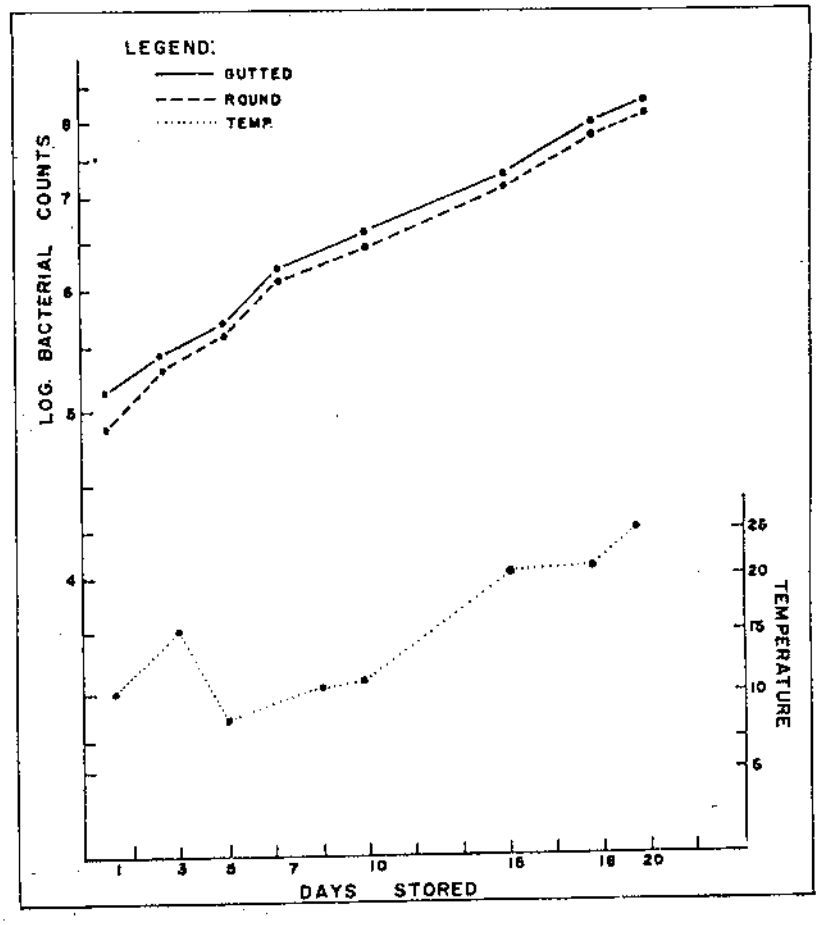
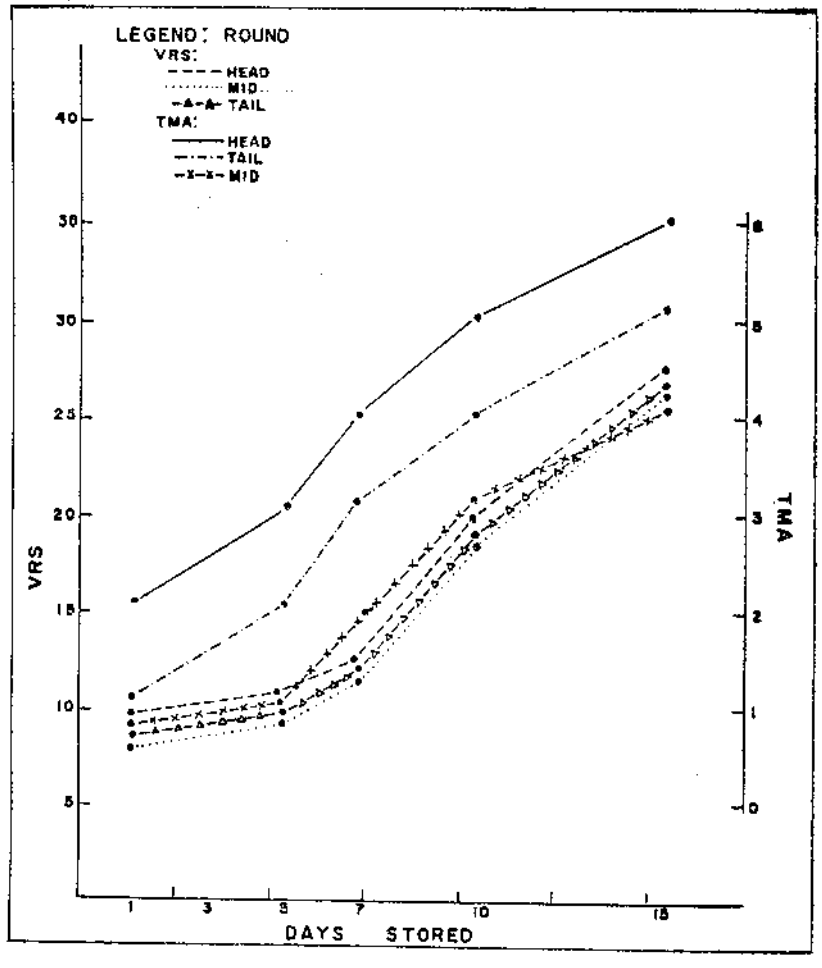


Fig. 2b. Changes in log bacterial count of round and gutted mackerel (*Auxis thazard* Lacepede) at varying storage period and temperature.

On the 20th day of storage (Fig. 2a) a considerable increase in TMA and VRS production was observed. The temperature of the compartment in the 20th day was 25.5°F. Gutted samples exhibited higher values, including bacterial count, than the round samples.

Organoleptic grading of fish samples revealed changes in odor, texture and appearance (Table 1) with increasing days of storage. Deterioration was also indicated by the presence of dis-



Figs. 3a and 3b. Variations in TMA and VRS of round and gutted mackerel during the storage period using head, mid and tail portions.

coloration and softening of belly cavity and the flesh, dull depressed eyes and loss of flesh bloom. Odors gradually changing from seaweedy to ammoniacal and putrid, also accompanied the chemical and bacterial reactions taking place during the final stages of decomposition.

The TMA and VRS tests were further applied in detecting highly imperceptible changes in quality. Round and gutted sam-

ples were divided into head, mid and tail portions and assessed by quantitative and bacteriological means.

Results in Figs. 3a and 3b (derived from Table 2) indicate that whether round or gutted, the head spoils more readily than the mid and tail, with the tail portion yielding higher values of

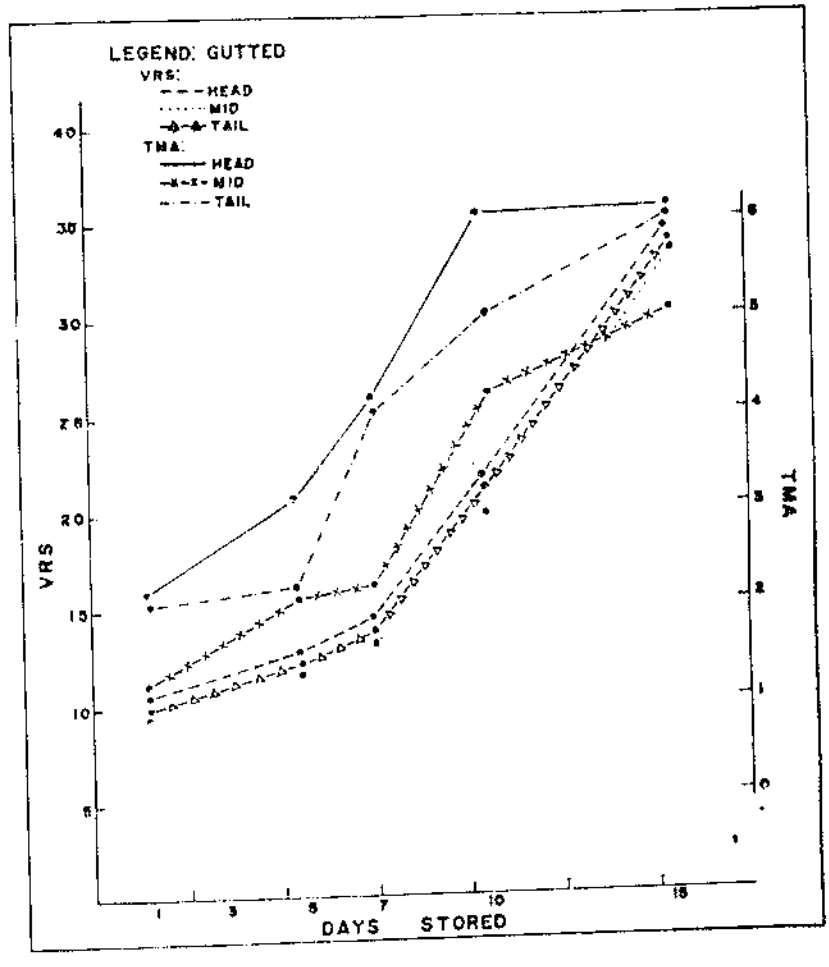


FIG. 3b.

TMA and VRS, next to the head. Bacterial counts made showed a similar pattern, using round and gutted samples (Tables 2c, Fig. 3c).

At 9.5°F the head of round samples yielded higher amounts of TMA and VRS on the first period of sampling. On the fifth

day of keeping, samples at a temperature of —8°F exhibited very slight increase of TMA and VRS. On the 5th and 7th day, samples of head, mid and tail; either round or gutted, were still considered relatively fresh. Round tail samples gave slightly higher values of TMA but not greater than that of gutted tail samples on the 7th day of sampling (Table 2).

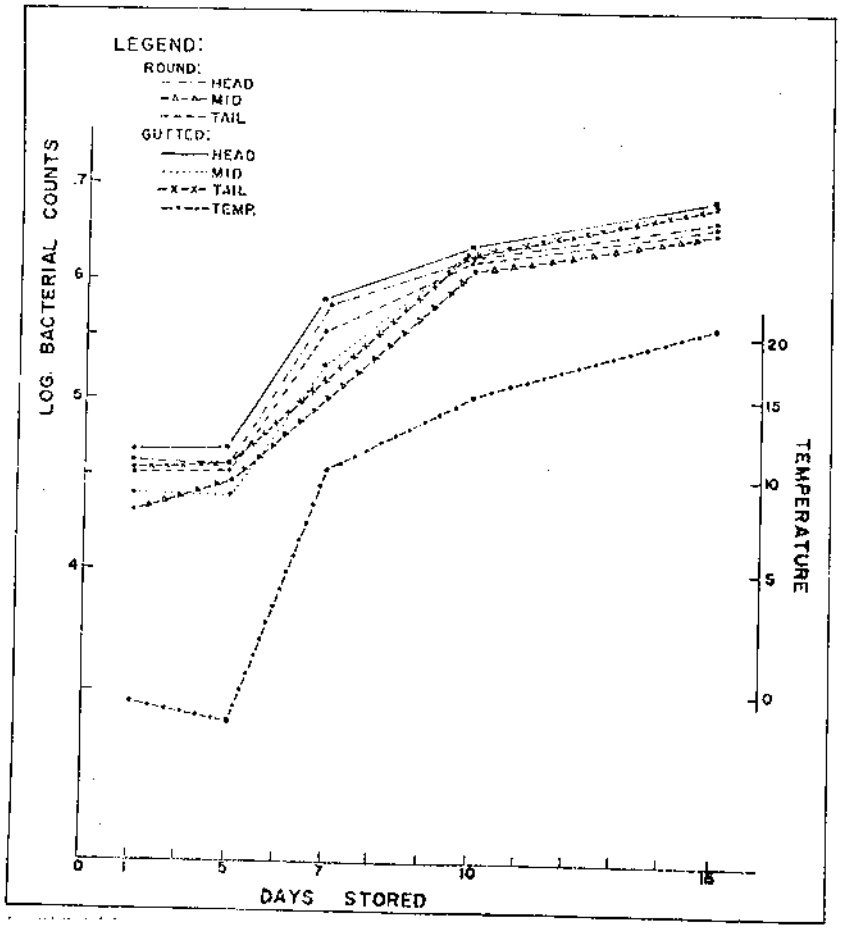


Fig. 3c. Variations in log bacterial counts of head, mid and tail portions from round and gutted mackerel, against storage time and temperature.

All samples whether round or gutted exhibited a considerable increase in TMA and VRS (Figs. 3a and 3b) as well as the bacterial count (Fig. 3c) and the samples first exhibited noticeable organoleptic changes in quality on the 10th day. A relatively

high temperature (15.2°F) was noted in the storage compartment.

VRS and TMA values ranging from 26.2 to 34.8 and 4.7 to 6.6, respectively (Tables 2a and 2b), were obtained from fish samples which appeared unacceptable on the 15th day of storage. The logarithm of bacterial plate counts ranged from 6.70 to 6.58 (Table 2c) at 20°F.

Results have shown that TMA and VRS values generally increased with rising bacterial counts. The occurrence of several chemical changes induced by bacterial and enzymatic decomposition which are in turn affected by temperature changes may generally account for the observed increased. The differences in TMA and VRS content between the head, mid and tail may be attributed to slight variations in chemical composition within the different parts of the fish (Stansby, 1961). Variations in the physical structure including the thickness of the flesh and the gross anatomical features of the head, mid and tail may also affect the rate of bacterial and enzymatic action, and consequently, VRS and TMA production.

Chemical tests for TMA and VRS seem to provide a means of assessing imperceptible changes in the state of quality of fish. Early intermediate stages of putrefaction may be detected accurately by using the chemical indices. It can be noted that this highly refined grading of quality cannot be achieved by organoleptic and bacteriological examinations alone.

A possible application of objective tests would be the evaluation of the market quality of fish. Preliminary tests were made to show the objectivity and applicability of the chemical tests. Samples from different markets were collected and chemically assessed periodically. Results showed relatively high values of TMA and VRS compared to newly caught control samples indicating that fish sold in the market have been insufficiently iced or stored for a long time before they reach the consumers. The above study on market quality assessment can be made a basis for more exhaustive work in the future.

Among the several factors which affect TMA and VRS production (Farber, 1956 and 1965) the preliminary processing treatment (either round or gutted, gilled or ungilled), is one of the factors to be considered in determining the rate of TMA and VRS development in the fish. Temperature is also very essential since the growth and activity of microorganisms and enzymes are

dependent on this factor. The higher the temperature, the faster is the development of TMA and VRS as well as bacteria.

The two chemical tests for freshness seem to be reliable yardsticks of quality. However, comparing the TMA and VRS values obtained, the former is quite low, making it difficult to indicate accurately the condition of the fish at the time when organoleptic signs of spoilage are hardly perceptible. Furthermore, TMA is formed only in a majority of marine fishes making the tests inapplicable to fresh-water fish (Anon., 1960).

The VRS test on the other hand can be utilized to assess the state of quality of all types of wet fish and fishery products (Farber, 1965). The VRS values are relatively higher than the TMA, making it possible to detect the earlier stages of decomposition. The Farber apparatus used can be assembled and dismantled without difficulty although an air-tight set-up must be maintained. On the contrary, the apparatus used in the TMA test is quite simple, consisting of a Conway unit with a removable cover. Volatilization of amines, however, may not be entirely eliminated due to the nature of the set-up.

The correlations between the VRS (Amos, 1962) and TMA (Castell *et al*, 1956) of fish samples with those of organoleptic standards, are indicated below:

For VRS:

0 to 10	fresh fish
11 to 20	first observable changes in quality including initial autolytic changes
> 20	later stages of autolysis and initial stages of putrefaction
> 30	for the later stages of putrefaction

For TMA:

0 to 1	fresh fish
2 to 5	Spoiling, under autolytic stages
> 5	later stages of putrefaction

In highly developed countries, these objective tests for freshness have been employed in the past decade in the assessment of keeping quality of fish and fishery products and have proven to be highly reliable and applicable (Burgess, 1965 and Farber, 1965).

Preservation studies using chemical additives, antibiotics (Tarr, 1956) and antioxidants and different processing methods may be properly evaluated with the use of these objective methods as a "yardstick" of keeping quality.

Locally, the chemical assessment of the quality of fish by TMA and VRS tests will most likely find several applications in the near future. In the fish canning industry for instance, where the freshness of raw material is foremost in importance in assuring products of good quality, objective methods will be of great advantage since fixed standards of quality could be more easily drawn than when using subjective or organoleptic tests. The market quality of fish and fishery products can also be assessed by objective methods and the chemical tests may be made use of in routine inspection of fish and fishery products sold in the market. The results can be made an objective basis for rejection or acceptance of commercial fishery products. The proper evaluation of local preservative methods can also be facilitated by the use of objective chemical tests and well-defined standards of quality.

REFERENCES

AMLACHER, E. (1961). Rigor Mortis in Fish. In Fish as Food. Borgstrom, ed. New York: Academic Press, vol. 1, 725 pp.

AMOS, A. J. *et al* (1965). Trimethylamine. In Food Industries Manual. London: Leonard Hill (Books), 1077 pp.

ANONYMOUS (1960). Freshness of Fish. Current Affairs Bulletin. FAO Indo-Pacific Fisheries Council, No. 27, p. 1.

ATILANO, J. (1965). A Chemical Method of Determining Spoilage in Fish. Undergraduate Thesis, College of Fisheries, University of the Philippines.

ATKINSON, K. (1965). A Chemical Test for Freshness of Fish. In Fish Handling and Processing. Edinburgh: Her Majesty's Stationary Office, 390 pp.

BURGESS, G.H. ed. (1965). The Measurement of Spoilage of Wet Fish. In Developments in Handling and Processing Fish. London: Fishing News Ltd., 132 pp.

CASTELL, C.H., R.S. RODGERS, and A.S. MCFARLANE (1956). Problems in Grading Cod and Haddock for Quality. I. Comparison of Organoleptic Grading With TMA Values. In Chilling of Fish. Hess, E. and G.N. Subba Rao, eds. Hague, Netherlands, 276 pp.

CASTELL, C. H., J. DALE, and M. GREENOUGH (1959). Spoilage of fish in the vessels at sea. Jour. Fisher. Res. Board of Canada 16 (2): 223-233.

CONWAY, E. (1962). Microdiffusion Analysis and Volumetric Error. London: Crosby Lockwood and Sons, Ltd., 367 pp.

FARBER, L.A. (1965). Review of the VRS Method for the Determination of Spoilage of Fish. In Technology of Fish Utilization, Kreuzer, ed., 280 pp.

FARBER, L.A., and A. CEDERQUIST (1953). The determination of volatile reducing substances as in quality control of fish products. Food Tech. 7: 478.

FARBER, L., and P. LERKE (1956). The Objective Assessment of Raw Quality. In Chilling of Fish. Hess, E. and G.N. Subba Rao, eds. Hague, Netherlands, 276 pp.

FRAZIER, W. (1965). Spoilage of Fish and Other Seafoods. In Food Microbiology. New York: McGraw-Hill Book Co., Inc., 472 pp.

JACOBS, M. (1651). The Analysis of Foods for Spoilage. In the Chemistry and Technology of Food Products. New York Interscience Publisher, 1: 952 pp.

PARTMANN, W. (1965). Changes During Rigor Mortis. In the Technology of Fish Utilization, Kreuzer, ed. London: Fishery News (Books) Ltd., 280 pp.

ROMERO, L. (1964). An Objective Quality Assessment of Raw Fish. Undergraduate Thesis, College of Fisheries, University of the Philippines.

SHEWAN, J.M., and L. LISTON (1956). Recent Work on the Use of Total Volatile Bases and TMA Contents and Tetrazolium Salt Reduction for Assessing the Quality of Iced Fish. In Chilling of Fish, Hess, E. and G.N. Subba Rao, eds. Hague, Netherlands, 176 pp.

STANSBY, MAURICE (1951). Proximate Composition of Fish. In Fish in Nutrition. International Congress. Washington, D.C., 447 pp.

TARR, H. L. A. (1956). Use of Preservatives and Antibiotics in the Preservation of Fresh Fish. In Chilling of Fish, Hess, E. and G.N. Subba Rao, eds. Hague, Netherlands, 276 pp.

YAMAGATA, M., K. HORIMOTO, and C. NAGAOKA (1971). Accuracy of Predicting occurrence of greening in tuna based on content of trimethylamine oxide. Jour. Food Sci. **36** (1): 55-57.

NEW AND NOTEWORTHY PHILIPPINE LIVERWORTS, II*

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National Museum, Manila

FIFTY-TWO TEXT FIGURES

ABSTRACT

Eleven species of primitive leafy liverworts are described. The three new species are *Herberta milleriana, Telaranea ponchoi,* and *Telaranea octoloba.* New records for the Philippines are *Acromastigum denticulatum* and *Microlepidozia gonyotricha.* Seven new combinations were made: *Bazzania luzonensis, B. halconiensis, B. elmeri, B. schadenbergii, B. cucullifolia, Telaranea semperiana,* and *Microlepidozia gonyotricha.*

The liverwort flora of the Philippines has received little attention since the start of botanical activity in the country. Of the approximately 10,000 species of liverworts described, only about 500 have been reported for the Philippines in spite of the botanical wealth of the country. Reports of collections date back to Montagne (1843) but the flora has remained little known. The considerable proportion of new forms and new records in all collections indicates that knowledge of our rich liverwort flora is very incomplete.

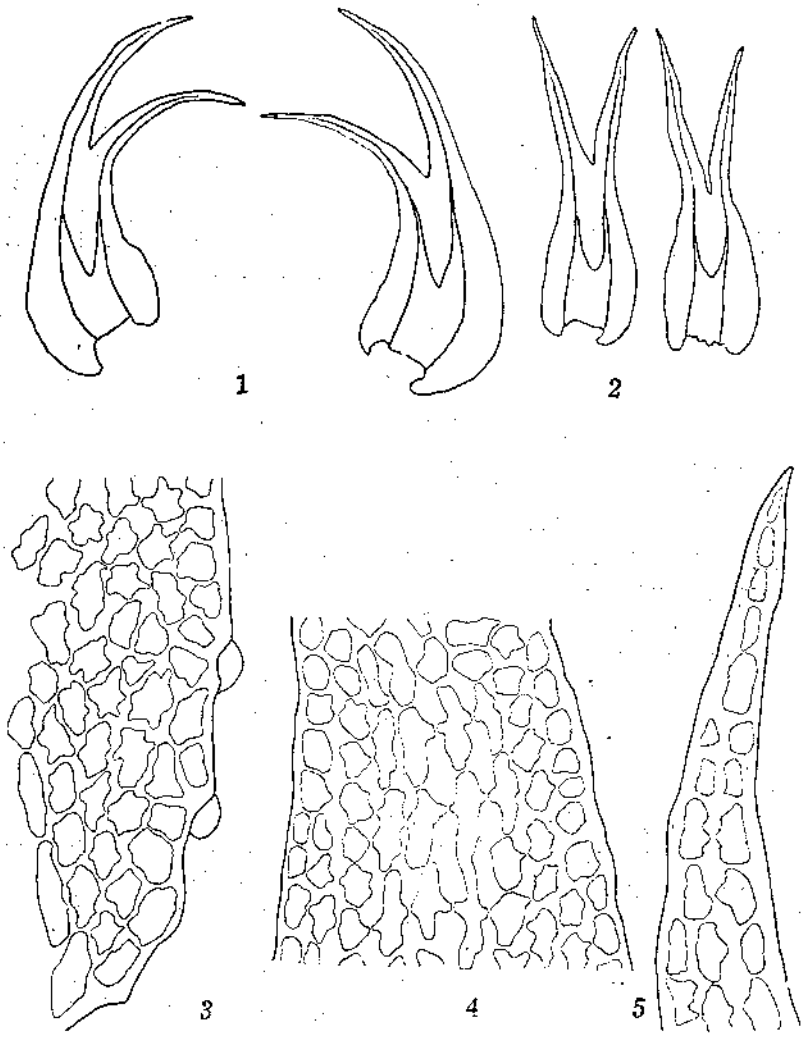
This paper reports 11 taxa, 3 of which are new species, 2 are new records, and 7 are new combinations. All these taxa with noteworthy morphology and distribution belong to the lower Jungermanniales *s. lat.* which is considered by Schuster (1966) and others to be the most primitive group of leafy liverworts except for the very small order Calobryales which is represented in the Philippines by a single species.

Material for this study was borrowed from the herbaria of Conservatoire et Jardin Botaniques at Geneva, Switzerland (G), the University of Michigan at Ann Arbor, Michigan (Mich), the College of Agriculture, University of the Philippines at Los Baños, Laguna (CAHUP), Philippine National Herbarium at Manila (PNH), and Dr. H. A. Miller (MH) who is with Florida Technological University.

HERBERTA MILLERIANA sp. nov. Figs. 1-5.

Folia caulina ovato-oblonga, falcato-secunda, discuss as mediam extensus; segmenta curva; trigonis magnis, nodulosis, non-

* No. I appeared in Philip. Jour. 95: 427-429, 1967. This paper is part of the dissertation submitted to the Faculty of the Graduate School of the University of Illinois in partial fulfillment of the requirements for the degree of Doctor of Philosophy.



Figs. 1-5. *Herberta milleriana*. *1*. leaves, × 45; *2*. under leaves, × 45; *3*. antical leaf base × 720; *4*. middle leaf cells, × 240; *5*. antical leaf segment, × 240. Based on Miller 10566.

dum confluens. Specimen typicum "Mt. Pulog, Benguet, leg. H. A. Miller 10566" in Hb. PNH et MH conservatum.

Plants robust, dark to golden brown. Stems 35 by 0.35 mm with scattered ventral intercalary branches, some typically leaved and others flagelliform. Leaves 3 to 3.5 by 0.8 to 1.2 mm, imbricate strongly falcate-secund, slightly decurrent, bifid about 1/2

with an acute sinus and lanceolate curved segments; basal disc broadly ovate-oblong to deltoid with margin nearly entire and few sessile slime papillæ; vitta strong, concave, weakly canaliculate below and extending below tip; leaf cells verruculose, with large nodular trigones, 1/2 to equal or larger than lumen. Underleaves similar to leaves but straight with narrower sinus and nearly symmetrical, the basal margin more undulate with mostly sessile slime papillæ. Dioicous. Male inflorescence terminal, bracts in 3 to 6 pairs, expanded at base and deeply concave but otherwise similar to leaves; bracteoles as in bracts. Female plants not seen.

LUZON; Benguet, Mt. Pulog, *Miller 10566* (type), *10442, 10468, 10564, 10581, 10674, 10707.*

The distinguishing characteristics of this species are the ovate-oblong to deltoid leaves and underleaves, the high basal discs, strongly falcate-secund leaves and the curved segments, and the large nodular trigones of the leaf cells which are almost equal or even exceeding the lumen.

The species differs from *H. chinensis* by its broadly ovate-oblong to deltoid leaves strongly falcate-secund, little decurrent base which is not clasping, and the large nodular trigones which are not as confluent as in the latter species.

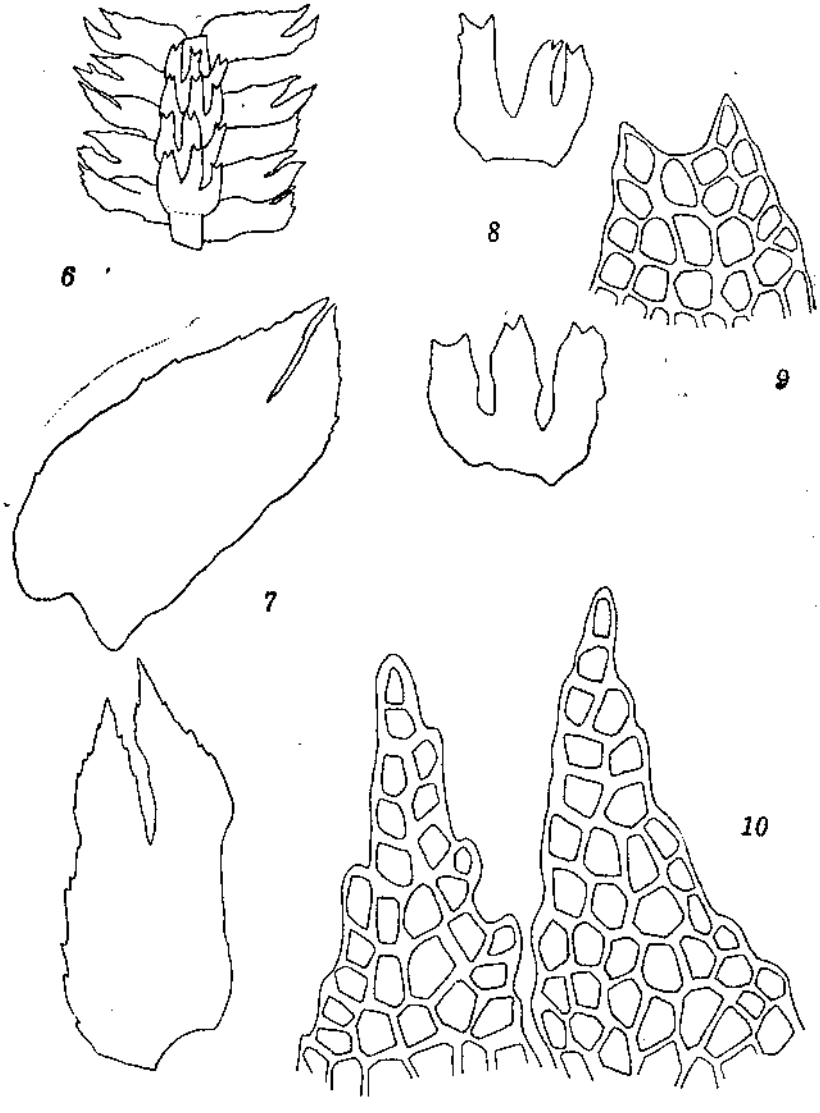
ACROMASTIGUM DENTICULATUM Evans. Figs. 6-10.

Acromastigum denticulatum EVANS, Ann. Bryol. Suppl. 3 (1934) 125.

Plants yellowish brown. Living portions 1 to 1.5 cm long, successive dichotomies 2 to 5 mm apart. Leaves usually imbricate, lying approximately in one plane, spreading obliquely or widely forming an angle of 60 to 80 degress, narrowly ovate-oblong, either straight or slightly falcate; dorsal base rounded and arched to about middle of axis; dorsal margin extends as an approximately straight line to apex of dorsal divisions; ventral margin subparallel with dorsal margin for half or 2/3 its length then curves gently forward; sinus 1/3 to 1/2 the length of leaves sharply to bluntly pointed at bottom both divisions narrowing gradually to sharp points and tipped with a single cell or with row of 2 cells, ventral division distinctly longer than dorsal, but may be just as narrow or narrower; margin irregularly crenulate or denticulate from projecting cells; vitta fairly well defined; leaf cells uniformly thickened without evident trigones. Underleaves loosely imbricate, orbicular-quadrate, deeply retuse or sharply bidentate, tooth composed of a single cell or 2 cells in a row, margin similar to those of leaves.

SAMAR, Borongan, *San Gabriel, Gutierrez,* and *Reynoso BS-67, BS-74* (PNH).

Distribution. - This species was previously known only from the type locality - Mt. Matang, Sarawak.

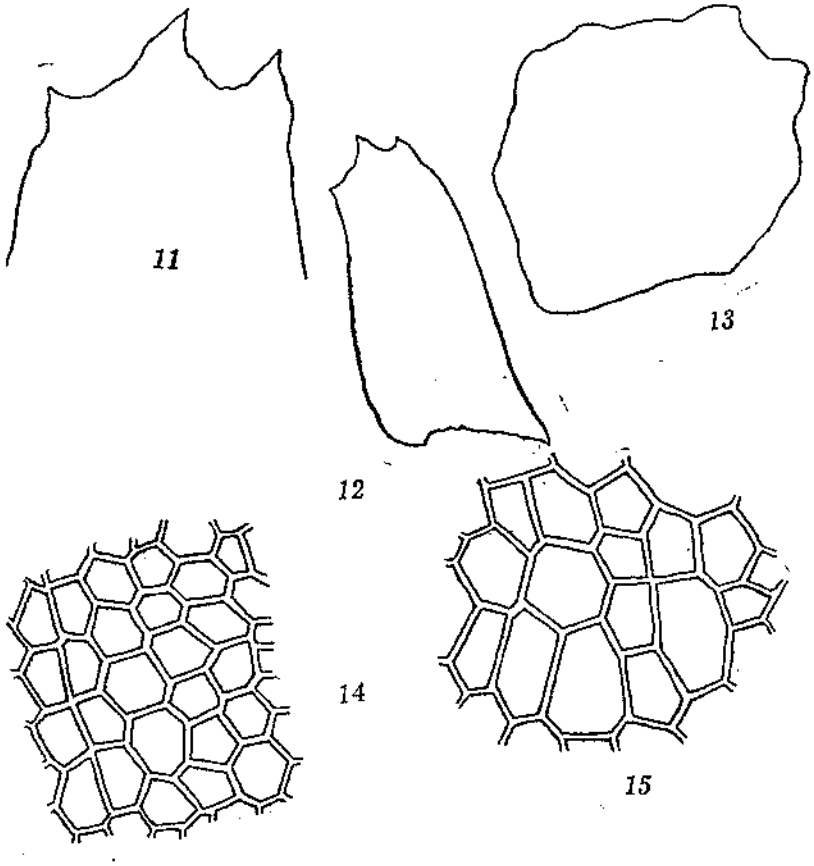


Figs. 6-10. *Acromastigum denticulatum*. *6*. portion of a plant, × 55; *7*. leaves × 127; *8*. underleaves, × 127; *9*. apical portion of underleaf lobe, × 750; *10*. apical lobes of leaves, × 750. Based on Gutierrez and Reynoso BS-67.

BAZZANIA LUZONENSIS (Steph.) comb. nov. **Figs. 11-15.**

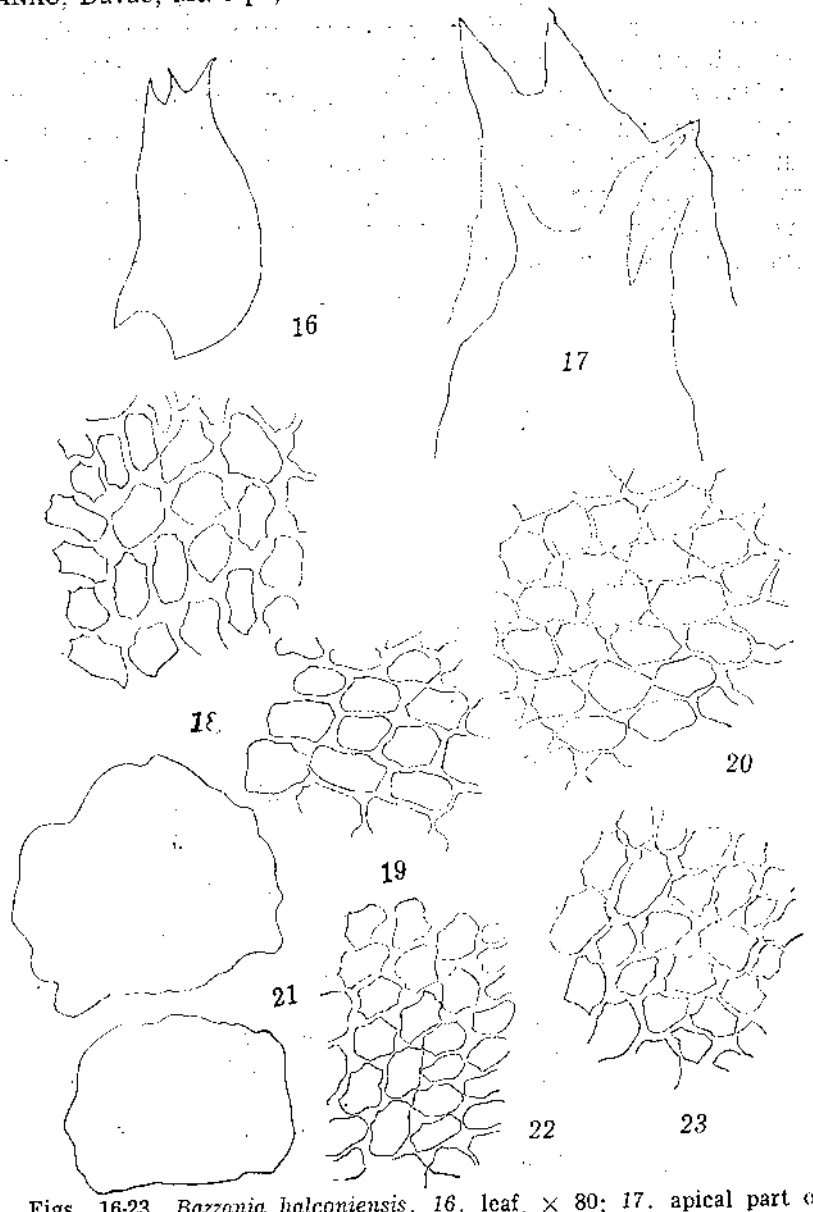
Mastigobryum luzonense Steph. Spec. Hepat. 6 (1924) 472.

Plants slender, flaccid, pale yellow to reddish brown. Stem reaching 7 cm long, ventral branches more frequently flagelliform; branches up to 3 cm long. Leaves slightly imbricate, more or less concave, narrowly oblong, subsymmetrical about 1 mm long, 0.45 mm wide, apex regularly tridentate; median tooth larger than others and broadly triangular; vitta extending well toward apex; trigones conspicuous only in vitta cells; cuticle mostly smooth, sometimes with striolations over vitta. Underleaves hyaline, distant, small, apex truncate, entire to finely serrulate.



Figs. 11-15. *Bazzania luzonensis*. *11*. apical part of leaf, × 127; *12*. leaf, × 30; *13*. underleaf, × 127; *14*. median leaf cells, × 750; *15*. median underleaf cells, × 750. Based on Robinson (type, G-14523).

LUZON, Tayabas, Infanta, *C. B. Robinson 9408* (G-14525), without locality, *9411*, type (G-14523), *9412* (G-14524), MINDANAO, Davao, Mt. Apo, *Miller 11004*.



Figs. 16-23. *Bazzania halconiensis*. *16*. leaf, × 80; *17*. apical part of leaf, × 127; *18*. apical leaf cells, × 750; *19*. basal leaf cells, × 750; *20*. median leaf cells, × 750; *21*. underleaves, × 127; *22*. upper underleaf cells, × 750; *23*. basal underleaf cell, × 750. Based on Merrill (type, G-10753).

This species differs from *B. vittata* by being larger, the ventral branches more frequently flagelliform, the leaves narrower with smooth cuticle and more yellowish, their apices more regularly bidentate, the vitta extending well toward the apex, and the more uniformly truncate apex of the underleaves.

An annotation of the type specimen indicates that the species was considered by Kitagawa to be a synonym of *B. intermedia*—probably because of the general features of the leaves as the tridentate leaf apex and the irregular serrulation toward the apex, but the latter are generally larger plants. Its leaves are much broader, being more ovate in outline, the vitta may extend beyond the middle but is not clearly defined and the trigones are more conspicuous than in the present species. The underleaves of *B. luzonensis* are smaller, semicircular and are distant while those of *B. intermedia* are larger, almost quadrate, more or less imbricate; and more irregularly lobed in their apices. Moreover; the chlorophyllose cells generally occupy a greater portion of the underleaf so that the hyaline border is well defined. *B. luzonensis*, is therefore, considered a valid species as regarded by Evans (1939) and Meijer (1960).

BAZZANIA HALCONIENSIS (Steph.) comb. nov. Figs. 16 - 23.

Mastigobryum halconiensis Steph. Spec. Hepat. **3** (1908) 443.

Plants very large; rigid; reddish-brown. Stem reaching 15 long, few-branched; branches slender; flagella long, numerous. Stem leaves 3.25 mm long and 0.7 mm wide, imbricate, decurved-homomallous, narrowly oblong, base 1.2 mm wide, apex 0.5 mm wide, slightly falcate apex oblique-truncate, tridentate, teeth narrowly triangulate, acuminate; upper leaf cells 21 by 15 μ; trigones large, nodulose; stem underleaves distant, slightly wider than stem, transversely inserted, appressed, subquadrate, entire to repand.

MINDORO, Mt. Halcon, *Merrill 6219* (type, G-10753).

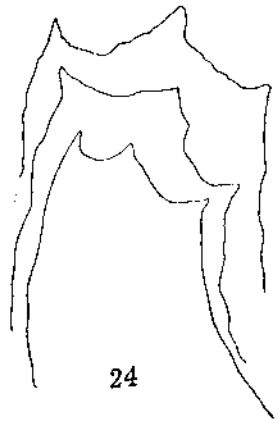
Distribution. – Endemic.

This species differs from *B. pectinata* by its sharp leaf lobes and the larger trigones in both the leaf and underleaf cells.

BAZZANIA ELMERI (Steph.) comb. nov. Figs. 24 - 28.

Mastigobryum elmeri Steph. Spec. Hepat. **6** (1924) 462.
M. mindanai Steph. *Ibid.* **6** (1924) 473.

Plants very large, rigid, reddish-brown. Stem reaching 15 cm long, repeatedly branched, branches 3 cm long; flagella long,



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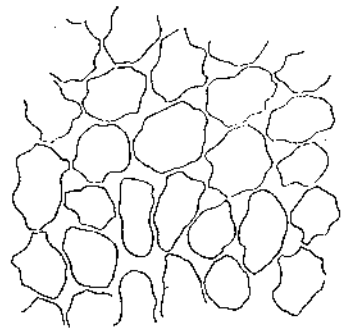

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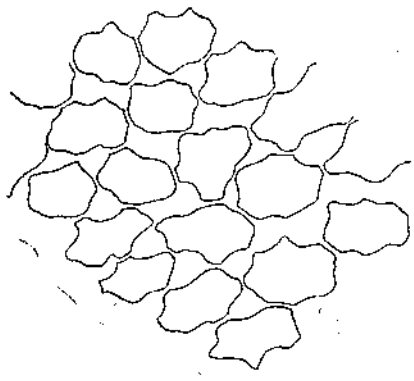

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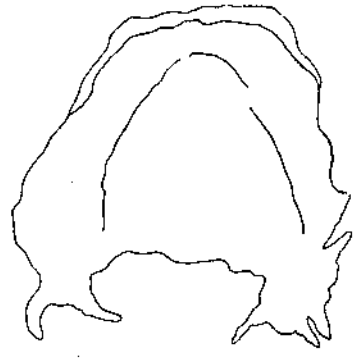

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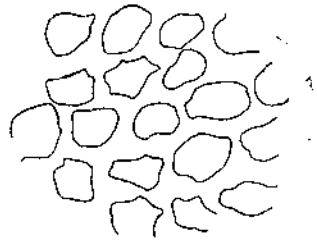

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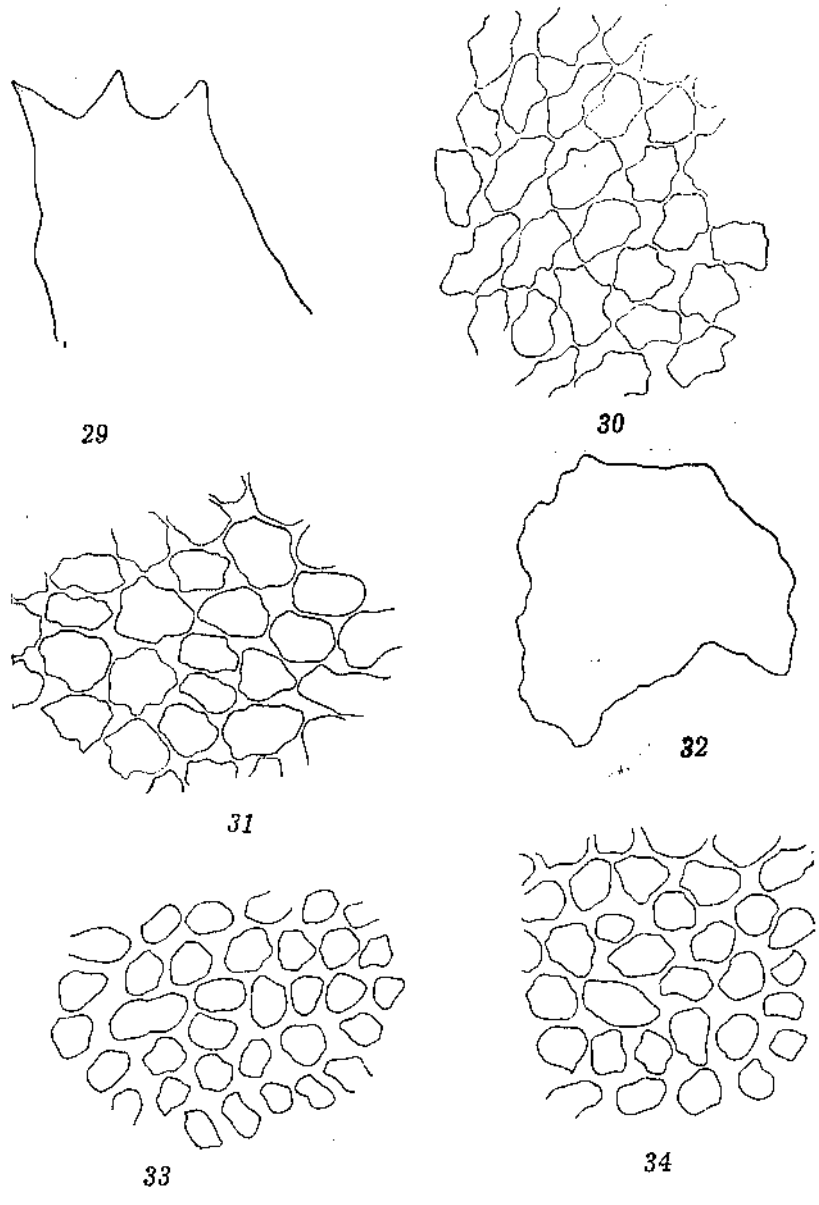
Figs. 24-28. *Bazzania elmeri*. 24. apical portion of leaves, × 127; 25. upper leaf cells, × 750; 26. median leaf cells, × 750; 27. underleaf, × 750; 28. under leaf median cells, × 750. Based on Elmer (type, G-14520).

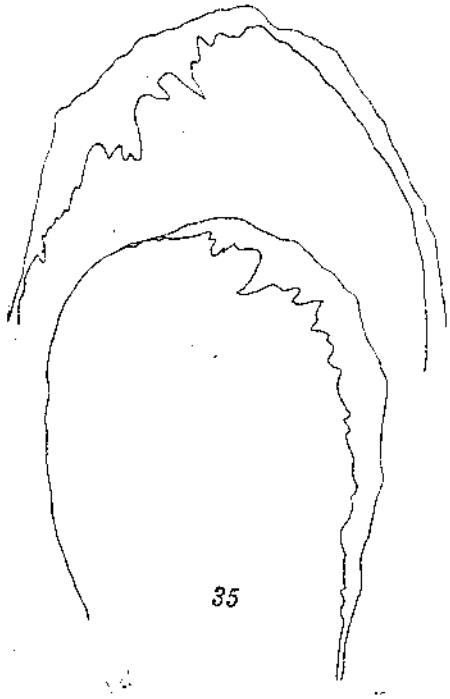
very numerous. Stem leaves imbricate, slightly concave, oblong, 1.8 mm long, 0.3 mm wide, tridentate; upper leaf cells 22 by 19 μ, trigones large, nodulose, always confluent. Underleaves subquadrate 0.5 mm long and wide, dentate, narrowly recurved, margin dentate, base appendiculate to subhastate.

MINDANAO, Agusan, Elmer, 1912 (type G-14520); Elmer, 1912 (type of *Mastigobryum mindanai* Steph. G-14529).



Figs. 29-34. *Bazzania schadenbergii*. *29*. apical portion of leaf, × 127; *30*. upper leaf cells, × 750; *31*. median leaf cells, × 750; *32*. underleaf, × 127; *33*. apical underleaf cells, × 750; *34*. basal underleaf cells, × 750. Based on Schadenbergi (G-14533).

This species somewhat resembles *B. gedeana* but is distinct in having conspicuous teeth on the ventral leaf bases and the narrower underleaves. In *B. gedeana*, there are no teeth on the



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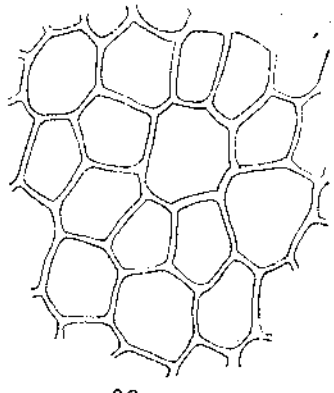

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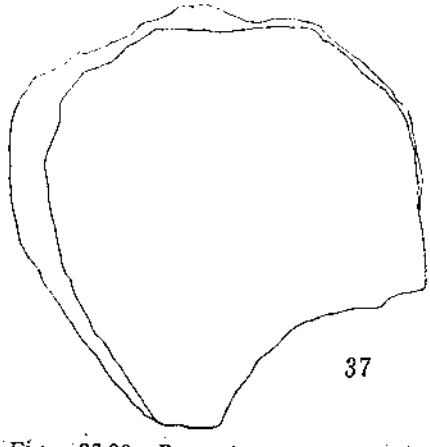

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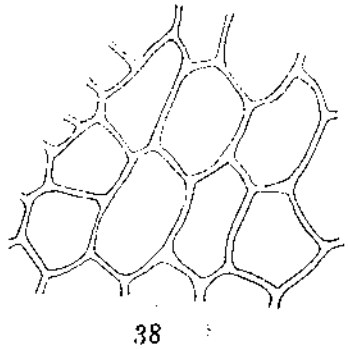

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Figs. 35-38. *Bazzania cucullifolia*. *35*. apical portion of leaves × 127; *36*. median leaf cells, × 750; *37*. underleaf, × 127; *38*. median underleaf cells, × 750. Based on Curran (G-12709).

ventral leaf bases and the underleaves are about three times as the stem.

BAZZANIA SCHADENBERGII (Steph.) comb. nov. **Figs. 29 - 34.**

Mastigobryum schadenbergii Steph. Spec. Hepat. 3 (1908) 515.

Plants yellowish-red, flaccid. Stem reaching 8 cm long, weak, irregularly branched; flagella long, filiform, numerous. Stem leaves 3 mm long, contiguous, straight spreading, slightly decurved, narrowy oblong, subsymmetrical, base 1 mm wide, apex 0.3 mm wide, normally constricted below the truncate tridentate apex; median tooth short, straight, lateral teeth widely divergent, upper leaf cells 25 μ with trigones, large, acute, basal cells 46 by 22 μ with trigones large and confluent; cuticle papillose. Underleaves distant, twice the stem width, appressed, subquadrate, subentire, base auriculate.

LUZON, Vigan, Schadenbergi, 1890 (type, G-14533); Manila, Semper (G-14534).

Distribution. - Java, Philippines.

I compared the type specimens of this species and that of *B. halconiensis* and found them to be very distinct from each other. *B. schadenbergii* differs from the latter species by its auriculate underleaves which are crenate to irregularly lobed, the lobes being divergent. In *B. halconiensis*, the underleaves are not auriculate, the margin is repand to irregularly toothed about one cell high and the leaves are deeply lobed, the lobes being less divergent than *B. schadenbergii*.

BAZZANIA CUCULLIFOLIA (Steph.) comb. nov. **Figs. 35 - 38.**

Mastigobryum cucullifolium Steph. Spec. Hepat. 6 (1924) 460.

Plants slender, flaccid, olive green. Stem 6 cm long, sparsely branched, flagella long, numerous. Leaves dense, straight, spreading, concave, triangular-ovate, 1.3 mm long, 0.8 mm wide at base, asymmetrical; apex narrowly decurved forming a hood with short teeth; dorsal margin arched toward base, ventral margin more or less upright; upper leaf cells 21 by 21 μ; trigones small or lacking; basal cells 36 by 60 μ; trigones small; marginal cells larger and thick-walled. Stem underleaves large, imbricate, circular-quadrate, entire, 0.7 mm in length and width.

NEGROS; without locality, Curran, 1909, *For. Bur. 13745* (type G-12709).

Distribution. - Endemic.

This is one of the beautiful and seemingly rare species of *Bazzania* in the Philippines. The wide suboblong leaves with the

toothed-hoodlike apex is the most distinctive feature of the species.

TELARANEA SEMPERIANA (Steph.) comb. nov. Figs. 39 - 42.

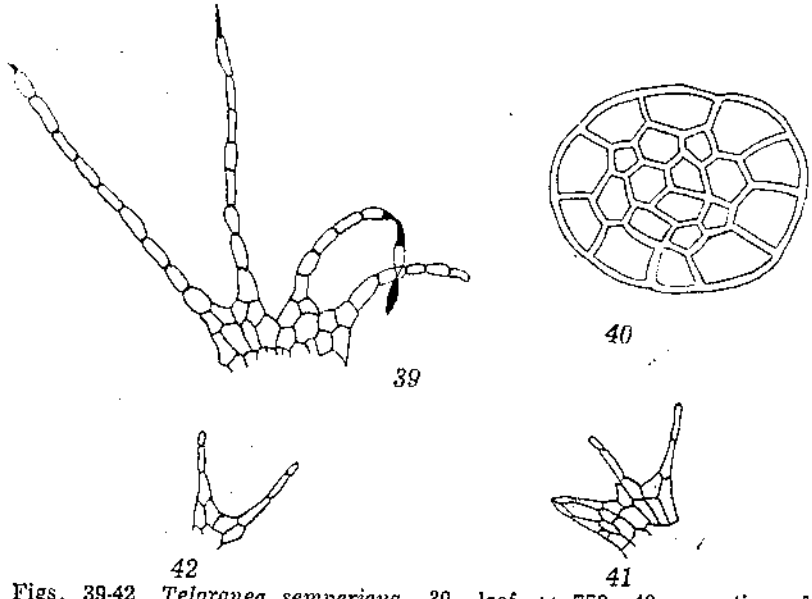
Lepidozia semperiana Steph. Spec. Hepat. 3 (1909) 612.

Plants slender, pale green. Stem 3 cm long, branches pinnate, short and distant; pinnæ straight and spreading, becoming attenuate, stoloniferous. Stem leaves somewhat plane, symmetrical, 4-lobed; lobes divergent, setose, 7 to 10 cells long, base 0.22 mm wide, 0.6 mm long. Cells in lobes 70 by 21 μ, in disc 49 by 28 μ; cuticle smooth. Underleaves similar to leaves but smaller and with setose lobes, 3 to 4 cells long. Branch under leaves bifid, 2 to 4 cells long.

LUZON, without definite locality. Semper (G-12707) type of *L. semperiana*.

MINDORO, Puerto Galera, *H. H. Bartlett 13624* (MICH).

Distribution. - Ceylon, Philippines.

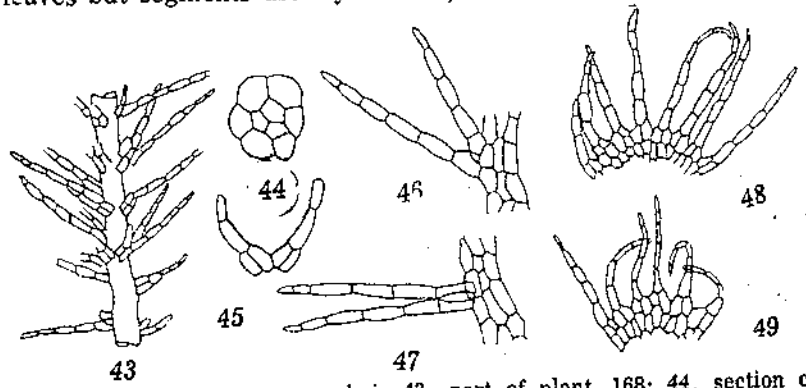


Figs. 39-42. *Telaranea semperiana*. *39*. leaf, × 750; *40*. x-section of stem, × 750; *41*. branch underleaf, × 127; *42*. stem underleaves, × 127. Based on Semper (G-12707).

TELARANEA PANCHOI sp. nov. Figs. 43 - 47.

Planta gracilis, folia et amphigastria profunde bifida cum uterque segmento cellularum 6 – 8 caulis cum hyalodermis 6 cellularis et medullar 3 – 4 cellularis. Specimen typicum "Laguna, Mt. Banahao, leg. J. V. Pancho 3303" in hb. PNH conservatum.

Plants small, filamentous, apple-green to whitish. Stems slender, filiform to 1 cm long, pinnate to bipinnate, lateral branches rarely becoming flagelliform; stem in transverse section with a unistratose cortex of 6 large cells surrounding 3 to 4 small cells. Line of leaf insertion transverse. Stem leaves delicate, spreading, of two long filamentous segments 6 to 8 cells long, or each segment from a 2-celled base, bases forming a disc of 4 cells across and half a cell high. Stem underleaves similar but smaller and shorter, 2 to 4 cells long. Branch leaves similar to leaves but segments usually shorter, 4 to 6 cells long.



Figs. 43-47. *Telaranea panchoi*. *43*. part of plant, 168; *44*. section of stem, × 720; *45*. stem underleaf, × 375; *46*. and *47*. stem leaves, × 375. Based on Pancho 3305.

Figs. 48-49. *Telaranea octoloba*. *48*. stem leaf, × 168; *49*. stem underleaf × 168. Based on Edano 605.

LUZON, Laguna, Mt. Banahao, *Pancho 3303* (typus, PNH).

The remarkable feature of this species is the presence of a few exceptionally large cortical cells seen in the cross section of the stem. The plants are extremely small, the leaves and underleaves are bilobed, each lobe with or without a 2-celled base, and the cuticle is smooth.

Figs. 48-49.

TELARANEA OCTOLOBA sp. nov.

Planta gracilis cum foliis in caulis denso; folium cum 8 segmentis uterque 6-8 cellularibus longis; stomium perianthiis cum ciliis simplicibus. Specimen typicum "Davao, Mt. McKinley, leg. E. Edano 605" in hb. PNH conservatum.

Plants small, yellowish green, filamentous. Stems slender, 1 to 2 cm long, densely leafy above, irregularly pinnate to bi- or tripinnate, cross section with a cortical layer of 22 large cells, lateral branches long and densely leafy. Line of insertion transverse. Stem leaves distant to approximate, rectangular to cuneate; 0.3

to 0.5 mm long, 0.4 to 0.6 mm wide, 8-lobed to 1/5 of their length, disc usually 2 cells high at margin, 3 to 4 cells high between, 16 cells wide, cells quadrate to rectangular, thin-walled; lobes uniseriate, usually 6 (sometimes 5 or 8) cells long, sometimes curved. Underleaves similar, usually 6-lobed, but lobes usually shorter. Branch leaves similar to underleaves. Female inflorescence on short ventral branch; bracts and bracteoles similar, lobed to 1/3 of their length. Perianth 4 to 5 mm long, cylindrical, mouth with cilia 4 to 6 cells long.

MINDANAO, Davao, Mt. McKinley, *Edano 605* (typus; PNH); Mt. Kampalili, *Edano 1096* (paratypus PNH).

Distribution. – Endemic.

The striking characteristics of this species are the densely leafy stem, the eight-lobed stem leaves, and the simple cilia present in the mouth of the perianth. The latter character distinguishes well this species from *T. neesii* wherein the cilia of the perianth are spiny and branched.

The genus *Telaranea* has not been reported for the Philippines. The distinguishing characteristics of the species belonging to the genus are: leaf insertion transverse, more than half of the symmetrical leaves divided into 4, 5 6, or 8 uniseriate filiform segments otherwise into 2, 3, or 4 uniseriate filiform segments often collapsed after drying, and the stem with well-marked hyaloderm.

With the transfer of *Lepidozia semperiana* to *Telaranea* and the recent collection of *Telaranea neesii* by Edano in Mt. Malbug, Negros Oriental, the genus has now 4 local species.

Key to the species of Telaranea

1. Stem leaves divided at base into 2, 3, or 4 uniseriate segments from a base of 2 cells; these 2, or 8 cells joined at least half their length to form a lamina one-half to 1 row of cells high; underleaves much smaller 2
   Stem leaves divided into 5, 6, or 8 uniseriate segments, often incurved; underleaves large and similar to leaves 3
2. Stems 2 to 5 cm long; stem leaves divided into 4 uniseriate segments, 8 to 21 cells long ..... *T. semperiana*
   Stems at most 1 cm long, delicate, stem leaves divided into 2 uniseriate segments ..... *T. panchoi*
3. Stem leaves usually divided into 6 uniseriate segments; cilia of bracts and perianth mouth with distinct spines and branches ... *T. neesii*
   Stem leaves usually divided into 8 uniseriate segments; cilia of bracts and perianth without spines ..... *T. octoloba*

MICROLEPIDOZIA GONYOTRICHA (Sande Lac.) comb. nov. Figs. 50 - 52.

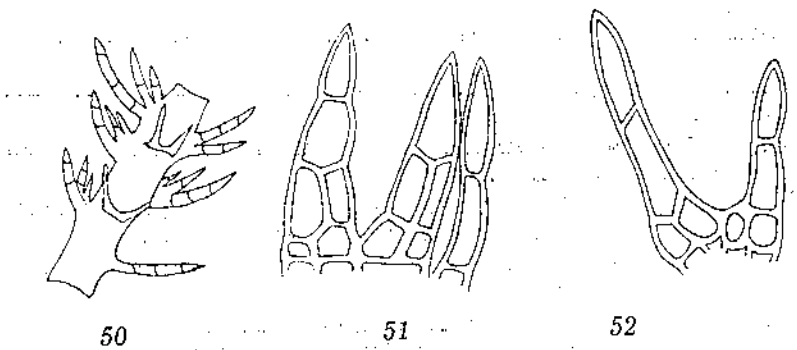
Lepidozia gonyotricha Sande Lac. Nederl. Druik. Archiv. **3** (1851) 521.

Kurzia crenacanthoidea v. MARTENS, FLORA N. R. **28** (1870) 417.

Lepidozia crenacanthoidea (v. Martens) GOEBEL, Ann. Jard. Buit. **9** (1891) 39.

Kurzia gonyotricha (Sande Lac.) GROLLE, Rev. Bryol. Lichenol. **32** (1953) 167.

Plants very small, filiform, yellowish-green. Stem up to 1 cm long slender, irregularly branched; branches distant, pinnate, occassionally bipinnate. Stem leaves minute, straight, spreading, divided into 3 or 4 segments; segments slightly divergent, 3 to 4 cells high; lamina a row of 6 to 8 cells broad; cells 8 to 15 μ by 30 to 36 μ; cuticle papillose. Underleaves much smaller than leaves, half as wide as stem, divided into 2 segments or 3 in robust plants; segments 1 to 2 cells high. Dioicous. Male inflorescence not seen. Female branches short, ventral intercalary in origin; bracts pale different from stem leaves, oval, irregularly divided into 4 ciliate segments; margin ciliate; perianth trigonous, mouth narrow, with longer, straight cilia; capsule not seen.



50 *51* *52*

Figs. 50-52. *Microlepidozia gonyotricha*. *50*. portion of plant, × 107; *51*. leaf, × 750; *52*. underleaf, × 750. Based on Pancho 3497.

NEGROS OCCIDENTAL, Mt. Canlaon, *Pancho 3497* (PNH).

Distribution. – Banca, Java, Moluccas, new to the Philippines.

This species differs from *M. makinoana* by its smaller size, the more symmetrical leaves and underleaves, the latter being mostly bilobed and the lobes being more or less equal. In the latter species, the leaves and underleaves are very symmetrical with the lobes more unequal.

ACKNOWLEDGMENT

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REFERENCES

EVANS, A. W. (1933). Some representative species of Bazzania from Sumatra. Pap. Mich. Acad. Sci. Arts & Letters **27**: 69-113.

EVANS, A. W. (1934). A revision of the genus Acromastigum. Ann. Bryol. Suppl. **3**: 1-178.

FULFORD, M. (1963). Segregate genera of the Lepidozia complex (Hepaticae. Part 2. Telaranea and a review of the Lepidoziaceæ. Brittonia **15**: 65-86.

GROLLE, T. (1963). Uber Kurzia v. Martens. Rev. Bryol. Lichenol. **22**: 166-180.

GROLLE, T. (1965). Lebermoose aus New Guinea. I. J. Hattori Bot. Lab. **28**: 43-54.

GROLLE, T. (1966). Lebermoose aus New Guinea. 5. Teleranea. J. Hattori Bot. Lab. **29**: 285-289.

HATTORI, S., and H. MIZUTANI (1958). A revision of the Japanese species of the family Lepidoziaceae. J. Hattori Bot. Lab. **19**: 76-118.

HERZOG, T. (1931). Hepaticae Philipinensis A. Cl. J. Baker Lectae. Ann. Bryol. **4**: 79-94.

HERZOG, T. (1932). Neue und Bemerkenswerte Bryophyten, von H. Burgeff 1927/1928 auf Java und den Philippinen gesammelt. Ann. Bryol. **5**: 29-32.

KITAGAWA, N. (1967). Studies on the Hepaticae of Thailand. I. The genus Bazzania with general introduction. J. Hattori Bot. Lab. **30**: 249-270.

MEIJER, W. (1960). Notes on the species of Bazzania (Hepaticae) mainly of Java. Blumea **10**: 367-384.

MILLER, H. A. (1965). A review of Herberta in the tropical Pacific and Asia. J. Hattori Bot. Lab. **28**: 299-412.

MIZUTANI, M. (1967). Studies of the Himalayan species of Bazzania. J. Hattori Bot. Lab. **30**: 71-90.

POCS, T. (1969). A short survey of Bazzania in North Vietnam. J. Hattori Bot. Lab. **32**: 79-94.

ROSARIO, R. M. DEL. (1967). Some liverworts from the Philippines. Bryologist **70**: 360-362.

SCHUSTER, R. M. (1966). The Hepaticæ and Anthocerotæ of North America, 1. New York & London: Columbia Univ. Press, 802 pp.

STEPHANI, F. (1900-1924). Species Hepaticarum. 6 vol. Genevé.

STEPHANI, F. Icones ineditae. Mastigobryum, Lepidozia. (Undated tracings of Stephani's unpublished drawings.

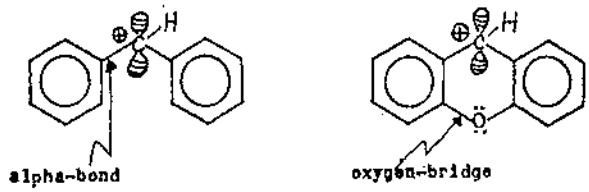
REACTIVE INTERMEDIATES IN RESEARCH, I
STABILITY OF BENZHYDRYL AND XANTHYL CATIONS*

By Bernadette I. Lazaro and Wendel Y. Lim[1]
University of the Philippines, Quezon City

ABSTRACT

Benzhydryl cation and its bridged analog, xanthyl cation, were prepared in concentrated sulfuric acid. The behavior of each species in this medium was studied by use of ultraviolet-visible spectroscopy and polarography. It is observed that xanthyl cation is more stable than benzhydryl cation. Results are explained in terms of recent electronic concepts.

Reactive intermediates are differentiated from transition states in that the former can be long-lived and can be conveniently studied [Kosower (1967), Leffler (1956), Olah (1970), and Wheland (1945)]. Reactive intermediates do not only exhibit interesting characteristic reactivity pattern of academic value but also features which are of great interest in industry and biology [Mahler and Cordes (1971)]. Planarity of some molecular intermediates has been one of the suspected criteria responsible for carcinogenicity. Benzylic systems, whether as neutral molecules or as intermediates are planar and do occur in nature as in some amino acids, pigments, hormones, etc. So it would be of great interest to study the nature of reactivity of two planar systems, where one is the "tied" form of the other. The benzhydryl group (chromophore in DDT, stilbisterol, etc.) and the xanthyl group (chromophore in actinomycins) were chosen to determine the effect of tying the two benzene rings together. This prevents their free rotation about the alpha-bond. An oxygen was chosen as this had not been well studied as a zero bridge as in structure



III (fluorenyl cation) or a carbon bridge (as in structure IV, anthranyl cation) where the bridge cannot participate in resonance due to its being tetrahedral or having an sp^3-type of hybrid-

* For previous paper in this series, see de Vera, N., and W. Y. Lim, Molecular complexes. Nat. Appl. Sci. Bull. **21** (1970), in press.

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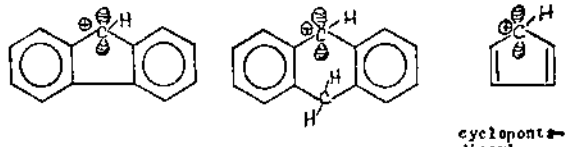
ization [Olah (1970) and Wheland (1945)]. Besides, earlier studies by Hine and co-workers (1967), in anionic systems, showed that oxygen is notorious in influencing the reactive site.



III IV V

In the benzhydryl cation, I, due to molecular vibration and rotation, there is a possibility that the two rings may not be coplanar with the carbonium ion center. This is hindered only if the ring participates in resonance or electron pi delocalization [Eyring *et al* (1944), Sylianco (1971), and Wheland (1945)]. The effect on the stability of the benzhydryl cation would then be predicted theoretically as due to electronic as well as statistical effects. However, one can eliminate the statistical factor by freezing the structure by the use of a bridge. The bridging can be attained by fusing the rings at the ortho-position as in III, by an oxygen atom as in II, by a methano bridge (methylene) as in IV, or perhaps by a sulfur or nitrogen atom. Fluorenyl cation has been well studied [Deno (1968), Fuson (1962), Gold and Bethell (1967), Hickinbottom (1957), and Streitweiser (1962)] and the stabilization of the cation has been found to be much less due to its antiaromatic character [Breslow *et al* (1970)] behaving like a cyclopentadienyl cation, V. The anthranyl cation has also been studied [Deno (1968), Eyring *et al* (1944), Fuson (1962), Gold and Bethell (1967), Hickinbottom (1957), Liberles (1968), and Streitweiser (1962)] but then the methano bridge can only participate as an electron-donating group by induction or hyperconjugation [Ferguson (1963)]; whereas, we would like to see the effect of a bridge which can provide resonance as in the system studied by Hine *et al* (1967). An oxygen bridge was decided and the two compounds, benzhydrol and xanthydrol were obtained and studied.

EXPERIMENTAL PART

Benzhydrol was synthesized from benzophenone by sodium amalgam reduction [Bachman (1953)] and subsequent purification by crystallization. Xanthydrol, which was obtained from Eastman Kodak Chemical Co., was purified using standard established procedure before use. The physical constants and infrared spectra

of the compounds were checked to confirm the identity and purity of the starting material.

A Bausch and Lomb (B & L) Spectronic 20 was used in the spectroscopic scanning of the cations. Before this publication, UV scan using the Beckman DU showed only refinements of the peaks but no significant alterations as compared with those obtained earlier with B & L model. An E.H. Sargeant Polarograph Model XXI was used in all polarographic scanning of the cations. A Perkin Elmer Infracord Model 137B was employed for all the IR spectra used in this study.

After screening for the most suitable concentration for UV analysis of the cations, it was observed that 10^{-2}M was the most convenient. To 2 milliliters of concentrated sulfuric acid were added 2 milliliters of the prepared reactant in absolute methanol. The solution was mixed uniformly for 30 seconds with the aid of a Lab-line Super Mixer. A complete scan of the spectrum from 340 nm[2] to 600 nm took about 30 minutes. Spectra were taken at different times after mixing, using intervals from 1 minute to 24 hours. All measurements were taken in an air-conditioned room where the temperature varied from 24 to 25°C only.

The polarographic measurements were determined using a standard calomel electrode as the reference. The capillary had an average drop-time of 2.5 seconds. The dissolved reactants and concentrated sulfuric acid were deaerated separately by a stream of purified nitrogen gas. Ten milliliters of the reactants in absolute ethanol were added to 10 milliliters of concentrated sulfuric acid. Varying concentrations of the reactants were also used (10^{-1} to 10^{-6}M). Dissolution was accelerated by fast stirring with a stream of nitrogen. Polarographic waves were recorded 5 minutes after mixing and every 30 minutes thereafter for several hours. Spectral test for the presence of the carbonium ion was done at the start of polarographic runs. The half-wave potentials were calculated from the polarograms using the procedure of Meites (1955).

RESUTLS AND DISCUSSION

Benzhydrol in concentrated sulfuric acid turned from white to yellow which intensified with time. This solution had been separately studied before [Deno (1968) and Gold and Bethell (1967)] and it was found to conduct electricity much more than pure sulfuric acid alone. These results were taken to mean that positive species existed in the solution. The maximum absorption

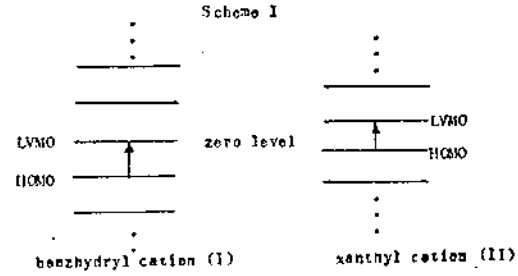
[2] nm means nanometer which is currently used in recent publications to replace μm (millimicrons).

in the UV-V region is shown in Table 1. After 1 minute, the spectra of benzhydrol showed a growing shoulder to the left of the maximum and persisted even after 24 hours while the maximum at 450 nm decreased. This could only mean that the carbonium ion was converted to products, possibly the bisulfates which would absorb in the shorter wavelength. Xanthydrol on the other hand absorbed at 370 nm which persisted even after 24 hours with no distinctive change in the spectra. Even if xanthyl cation were found to be long-lived, its absorption at the shorter wavelength was hard to explain. The absorption maximum of benzhydrol at 450 nm and that of xanthydrol at 370 nm, were confirmed to match with those values obtained in the literature [Deno (1968) and Gold and Bethell (1967)].

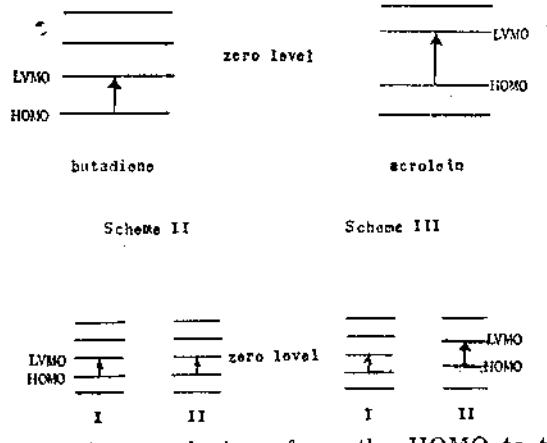
TABLE 1.—*UV-V spectra of xanthyl and benzhydryl cations.*

cationic species	λ-max (nm)	Δ_E kcal/mole	ϵ	time after mixing (min)
benzhydryl	450	63.6	1,170	1
xanthyl	370	77.4	17,100	1

The position of absorption in the ultraviolet-visible (UV-V) region is indicative of the gap or difference of the energy levels involved in the electronic excitation (from the highest occupied molecular orbital or HOMO, to the lowest vacant molecular orbital or LVMO). The extinction coefficient moreover can be used as a measure of the probability of exciting an electron from a HOMO to a corresponding LVMO. The probability does not usually go in the same direction as the energy requirement which is determined by the maximum position of absorption [Higasi *et al* (1965)]. The benzhydryl cation, I, is an odd-alternant species and therefore would behave like a benzyl cation [Liberles (1968) and Streitweiser (1962)] where one of the molecular orbitals (MO) falls on the zero energy level (or nonbonding MO). A xanthyl cation, II, on the other hand, has an additional oxygen in the structure. It should be treated as an even-membered species comparable with the anthryl cation. This system has no MO which falls on the zero energy level. It has been observed further that the MO levels will be brought closer together when the number of MO levels increases. If this case were followed, the energy requirement for the xanthyl cation, II, would be lesser than the benzhydryl cation and consequently manifests as an absorption in the longer wavelength. This is so since there is an inverse relationship between energy and wavelength of absorption (Scheme I).



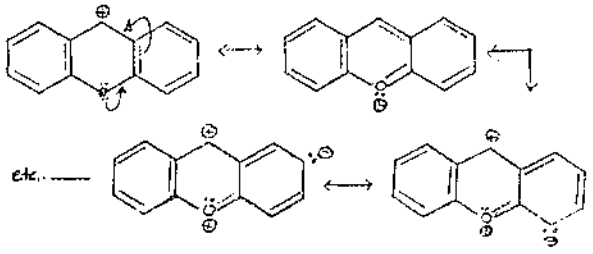
Another theoretical possibility would be to assume that the oxygen in xanthyl cation would have no effect and thus both cations would behave like odd-aternant systems. This would result in the same energy requirement hence the same maximum position of absorption in the UV-V. However, this second scheme was not observed. Unlike the first scheme and the second scheme, the observed trend (Table 1) was that the xanthyl cation absorbed 80 nm lower than the benzhydryl cation corresponding to a difference of 14.2 kilocalories per mole. It is imperative therefore to invoke a third scheme (Scheme III) to explain the experimental observation. Existing electronegative atoms perturb molecular orbital levels in such a way as to spread the gap between the HOMO and the LVMO [Roberts (1961)]. A good example to prove this point is by comparing 1,3-butadiene and acrolein [Roberts (1961)] as shown in Scheme IV. More energy



is required to excite an electron from the HOMO to the LVMO of acrolein than that of 1,3-butadiene. From the structural formula of these compounds, these only vary at the fourth atom. Instead of an oxygen at the fourth position, a carbon is found in

the 1,3-butadiene. The difference between carbon and oxygen is only in the electronegativity value for each atom. Oxygen being more electron attracting than carbon has a greater capacity to perturb the molecular orbital levels; hence, the spreading out of the HOMO from the LVMO. The increase in the energy gap between the HOMO and the LVMO is due to the destabilization of the excited state (when the electron has been excited to the LVMO) which has increased positive charge by the strongly electronegative atom. An electron donating atom or group would show an opposite effect [Higasi *et al* (1965)]. The next consideration would be the extinction coefficient. The trend is just the opposite to that of the wavelength of absorption. The greater the extinction coefficient, the greater is the probability for electrons to get excited from the HOMO to the LVMO. From Table 1 it can be inferred that there is more *pi*-electron delocalization in the xanthyl cation than in benzydryl cation. The electrons in the former are more delocalized or loosely held by the molecular framework in spite of the presence of an electronegative oxygen. Ordinarily, neutral molecules with highly delocalized MO's, tend to absorb in the longer wavelength. Our case is one of the exceptions [Ferguson (1963) and Higasi *et al* (1965)].

The more long-lived xanthyl cation therefore can be presented as follows:



Although resonance takes place, the electron-attracting effect of the oxygen still operates, and this is specifically felt in the excited state where the charges (positive) are increased due to electronic excitation.

Since cations are positive species, these should be electroactive and can easily be studied by use of polarography where electron transfer occurs. When both cations were studied as to their polarographic behavior; it was observed that considering concentration and time of mixing constant, xanthyl cation showed a less negative half-wave potential than benzhydryl cation (Table 2).

TABLE 2.—*Half-wave potentials of xanthyl and benzhydryl cations.*

Alcohol concentration M	Time of mixing min.	I $E_{\frac{1}{2}}$, v	II $E_{\frac{1}{2}}$, v
10^{-6}	5	—.84	—.79
	30	—.97	—.99
10^{-5}	5	—.85	—.80
	30	—1.01	—.99
10^{-4}	5	—.89	—.82
	30	—1.03	—1.02

The greater ease for polarographic reduction of xanthyl cation as compared with benzhydryl cation could only be due to the presence of oxygen. It is exerting its electronegativity effect across sigma bonds which is felt by the positive reactive site, thereby causing an ease of reduction.

So even if in xanthyl cation the oxygen participates in resonance stabilization of the cation, it also exerts its electronegativity effect as observed in the polarographic study.

Extended studies on the silylation rates of the cations and the inclusion of related heteroanalogs are being conducted. Such compounds as fluorenol, anthranol, the sulfur and nitrogen analogs of xanthydrol and the ketone analog are being considered.

ACKNOWLEDGMENT

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REFERENCES

BACHMAN, W. E. (1953). The mechanism of reduction by sodium amalgam and alcohol. Jour. Amer. Chem. Soc. **55**: 770.

BRESLOW, R., R. GRUBBS, and S. MURAHSHI (1970). Electrochemical evidence for the antiaromaticity of cyclobutadiene. Jour. Amer. Chem. Soc. **92**: 4139; and references cited therein.

DENO, N. C. (1968). Carbonium ions. Chem. Eng. News **45**: 88; (1964). Prog. Phys. Org. Chem. **2**: 127.

EYRING, H., J. WALTER, and G. E. KIMBAL (1944). Quantum Chemistry. New York: John Wiley & Sons **1**: 394 pp.

FERGUSON, L. N. (1963). The Modern Structural Theory of Organic Chemistry. New York: Prentice-Hall, 599 pp.

FUSON, R. C. (1962). Reactions of Organic Compounds. New York: John Wiley & Sons, 765 pp.

GOLD, V., and D. BETHELL (1967). Carbonium Ions—An Introduction. London: Academic Press, 3 pp; (1958). Quart. Rev. **12**: 173.

HICKINBOTTOM, W. J. (1957). Reactions of Organic Compounds. London: Longmans, Green & Co., 449 pp.

HIGASI, K., H. BABA, and A. REMBAUM (1965). Quantum Organic Chemistry. New York: Interscience Publishers Inc, 358 pp.

HINE, J., L. G. MAHONE, and C. L. LIOTTA (1967). *Alpha*-Fluoro and *alpha*-alkoxy as deactivators in carbonium formation. Jour. Amer. Chem. Soc. **89**: 5911.

KOSOWER, E. M. (1967). Introduction to Physical Organic Chemistry. New York: John Wiley & Sons, 503 pp.

LEFFLER, J. E. (1956). The Reactive Intermediates of Orbanic Chemistry. New York: Interscience Publishers, Inc., 275 pp.

LIBERLES, A. (1968). Introduction to Theoretical Organic Chemistry. New York: MacMillan Co., 722 pp.

MAHLER, H. R., and E. H. CORDES (1971). Biological Chemistry. New York: Harper & Row Publishers, 872 pp.

MEITES, L. (1955). Polargraphic Techniques. New York: Interscience Publishers, Inc., 481 pp.

OLAH, G. A. (1970). Stable carbonium ions. Science **168**: 1298; and references cited therein.

OLAH, G. A., and C. U. PITTMAN (1965). Carbonium ions. Advan. Phy. Org. Chem. **4**: 303.

ROBERTS, J. D. (1961). Notes on Molecular Orbital Calculations. New York: W.A. Benjamin, Inc., 156 pp.

STREITWEISER, A. (1962). Molecular Orbital Theory for Organic Chemists. New York: John Wiley & Sons, 489 pp.

SYLIANCO, C. Y. L. (1971). Principles of Organic Chemistry. Quezon City: Aurum Enterprises, 463 pp.

WHELAND, G. W. (1945). The Theory of Resonance and Its Applications to Organic Chemistry. New York: John Wiley & Sons, 316 pp.

ELECTRONIC AND STRUCTURAL EFFECTS ON RATES AND EQUILIBRIA, III. SOLVENT AND SUBSTITUENT INFLUENCE IN DEHYDROGENATION WITH QUINONES*

By Raul C. Oeach and Wendel Y. Lim[1]
University of the Philippines, Quezon City

TWO TEXT FIGURES

ABSTRACT

Using three different quinones, the dehydrogenation of 9, 10-dihydroanthracene was carried out individually in four separate solvents. The reactivity pattern of the quinones was observed to follow the order: 2,3-dichloro-5,6-dicyanobenzo-quinone (DDQ) > 2,3,5,6-tetrachlorobenzoquinone (chloranil) > 2,5-dehydroxy-3,6-dichlorobenzoquinone (chloranic acid). The sequence of reactivity in different solvents for each quinone followed the trend: ethanol >n-amylalcohol > chlorobenzene > 1,4-dioxene. Rate constants were determined for each case and results are explained in terms of polar reaction mechanism. Attempts to utilize the above results in the conversion of nonconjugated unsaturated fatty esters of the oil from *Aleurites moluccana* (Linn.) or lumbang, to highly polyunsaturated and conjugated esters, showed low conversion.

In our study of the chemistry of some natural products we observed that dehydrogenation in general is indispensable and yet it is one of the less studied reactions currently discussed in the literature [Hendrickson *et al* (1970) and March (1968)]. We have been doing catalytic and quinone dehydrogenations of the oil obtained from *Aleurites moluccana* (Linn.) and 9,10-dihydroanthracene. We have observed that the mechanism of quinone dehydrogenation, which had been studied before [Bailey (1951), Barnard and Jackman (1960), Braude *et al* (1960), Brown and Jackman (1960), and Charton (1964)], still remain unresolved.

Homolytic or free radical mechanism has been proposed by Moore and Waters (1953). This was based mainly on their work on photochemically-induced quinone dehydrogenation which could proceed by a free radical mechanism [Braude *et al* (1960), Hen-

* For the previous paper in this series, see Bun-Pok Ku and Wendel Y. Lim, Philip. Jour. Sci. **100** (1971) 115-129.

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drickson *et al* (1970), and March (1968)]. Molecular and polar reaction mechanisms were also proposed [Braude *et al* (1960)].

Considering the information gleaned from the literature and from the results of the preliminary work undertaken by our colleagues in the laboratory, we embarked on the study of the influence of solvent and substituent on the reaction in question. This aspect was chosen since polar reactions are sensitive to solvent polarity and substituent effect [Huheey (1965) and Jaffe (1953)], whereas, free radical and molecular reactions are not.

EXPERIMENTAL PART

The reactants 9,10-dihydroanthracene, 2,3,5,6-tetrachlorobenzoquinone (chloranil) and 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) were obtained from Aldrich Chemical Co., and 2,5-dihydroxy-3,6-dichlorobenzoquinone (chloranilic acid) was procured from J. T. Baber Chemical Co. Reagent grade 1,4-dioxane was purchased from Mallinckrodt Chemical Works; chlorobenzene and n-amyl alcohol from British Drug House; Ltd. and absolute ethanol from Ajax Chemical Ltd. All reactants were purified using standard procedures.

Melting points were determined using a Hoover Capillary Melting Point Apparatus. Ultraviolet spectral determinations during the kinetic runs were made with a Beckman DU Spectrophotometer, while a Perkin Elmer Model 137B Infracord was used for all infrared spectral analyses.

Dehydrogenation reaction.—To a solution of 1 mole of 9,10-dihydroanthracene in 80 ml of solvent in a three-necked round-bottomed flask under nitrogen atmosphere was added 1 mole of the quinone in 70 ml of the same solvent. The reaction mixture was allowed to reflux under nitrogen atmosphere. Aliquots were taken at definite time intervals. The aliquot was quenched by freezing and was analyzed for the disappearance of the quinone by an ultraviolet spectrophotometric method [Jaffe and Orchin (1962) and Silverstein and Bassler (1965)].

DISCUSSION OF RESULTS

The quinones were observed to follow Lambert-Beer's Law at the concentrations employed in this study. Sample curves are shown in Figures 1 and 2. The maximum absorption (λ max)

and molar extinction coefficient (ϵ) were determined and correlated with the property of the solvent. Dielectic constant was used, as suggested by Kosower (1967), as the parameter to characterize solvating power (Table 1).

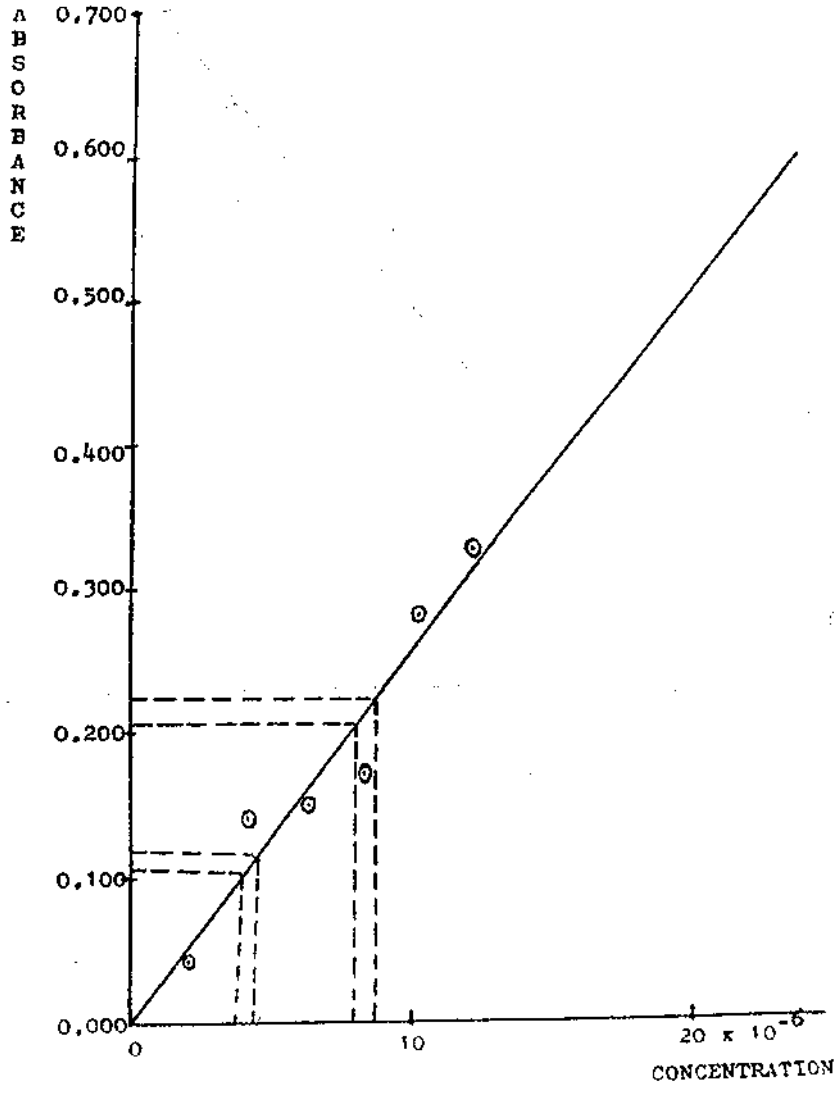

FIG. 1. Standard curve: chloranil in 1, 4-dioxane.

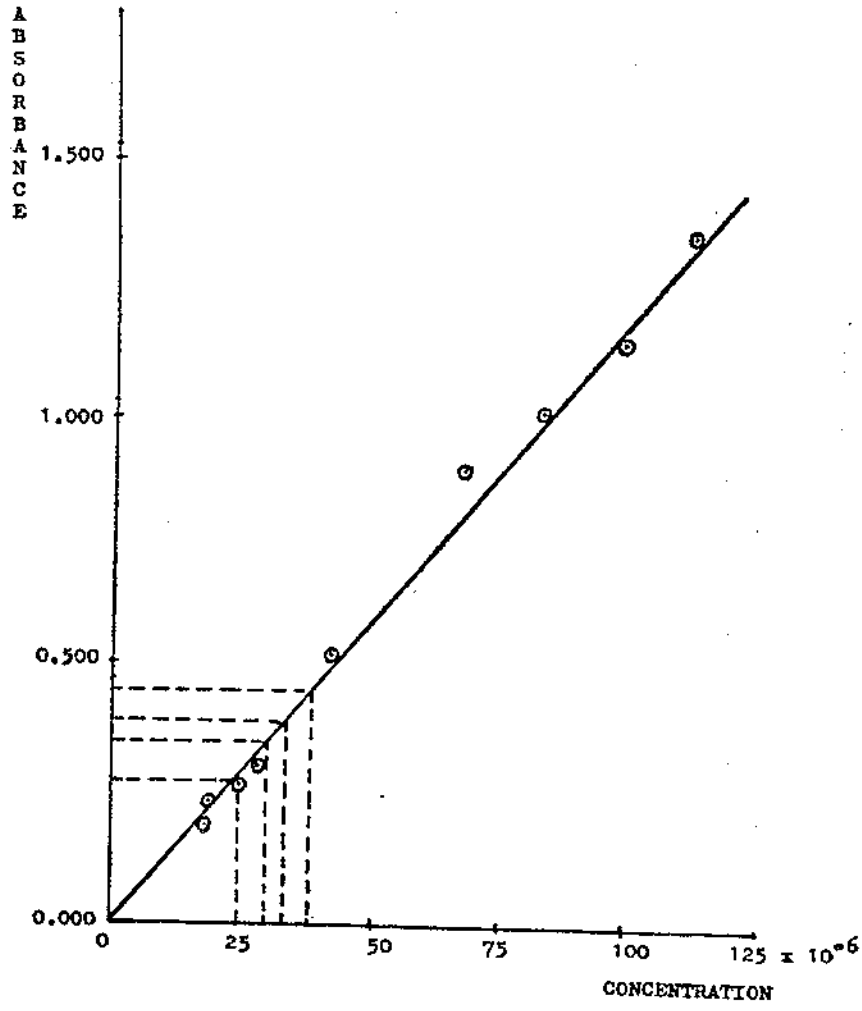

FIG. 2. Standard curve: chloranil in ethanol.

TABLE 1.

Quinone	Solvent	dielectric constant (D) of solvent	λ max nm*	ϵ ext. coef.
DDQ	dioxane	2.209	282	12.000
Chloranil	dioxane	2.209	285	22.940
Chloranilic acid	dioxane	2.209	305	17.450
Chloranil	chlorobenzene	5.620	287	12.130
Chloranil	n-amyl alcohol	13.90	288	17.050
Chloranil	ethanol	25.6	288	11.520

*nm means nanometer or 10^{-9}meter

For the same solvent, it is indicated in the above table that there is a progressive increase of wavelength of absorption, in the order: DDQ > chloranil > chloranilic acid. Wavelength is related to the energy required to excite an electron from the highest occupied molecular orbital (sometimes called the HOMO), to the lowest vacant or unoccupied molecular orbital (sometimes known as the LVMO) [Cavenaugh and Dailey (1961), and Jaffe and Orchin (1962)]. If it is assumed that there is no substituent effect in the quinones studied, then the energy gap between the LVMO and the HOMO for the three quinones would be the same. The manifested wavelengths would then be equal. This was not observed. The values as indicated in Table 1 show that the energy gap between the LVMO and the HOMO for DDQ is larger than that for chloranil, and for chloranil than the value for chloranilic acid. The difference could be attributed to the influence exerted by the different functions bonded to the quinone ring. These functions perturb the energy levels differently since the electronic capabilities of each substituent vary. There are only three types of substituents involved in this study: the cyano, chloro and hydroxy groups. The electronic features of these substituents have been established through what is known as the Hammett substituent constants [Charton (1964), Hammett (1971), Jaffe (1953), and March (1968)] and group electronegativity [Cavenaugh and Dailey (1961), Charton (1964), Dailey and Schoolery (1955), and Hinze *et al* (1963)].

TABLE 2.

Substituent	σ_I	σ_m	σ_p	E.N.*
CN	0.58	0.56	0.66	2.49
Cl	0.47	0.37	0.23	3.19
OH	0.25	0.12	—0.37	3.51

*-References [Cavenough and Dailey (1961), Charton (1964), Dailey and Schoolery (1955), and Hinze *et al* (1963)].

The cyano group is more electron-attracting than chloro or hydroxy group as shown from the substituent constant σ_I (inductive substituent constant), σ_m (*meta* substituent constant) or σ_p (*para* substituent constant) [Charton (1964)]. The last two sets of constants include resonance contributions in addition to pure inductive effects [Hine (1962)]. From examination of the group electronegatively values (E.N.), it can be observed that the trend is just opposite to that of the substituent constants. Group electronegativity then cannot explain the results obtained in this study. Group electronegativity; as presented, measures the predominant contribution of the bonding atom to the quinone ring

of the different functions. The order of atomic electronegativity is: O > Cl > C and in group electronegativity it is: OH > Cl > CN. The inadequacy of using group electronegativity to explain substituent influence stems from the method of determining this value. Many of the operations employed determine the electron density at the bonding atom [Cavenaugh and Dailey (1961), Dailey and Schoolery (1955), and Hinze *et al* (1963)] showed that this property does not necessarily represent the additivity and cooperatively of all the atoms present in the function. The cyano group is the most electron attracting substituent when bound to an aromatic or similar rings. This is due to the increased electronegativity of the carbon (50 per cent *s* character) and the presence of nitrogen which also has a digonal (sp) type of hybridization. Of the three substituents, the chloro and hydroxy groups have lone pairs on the bonding atoms. These lone pairs can be donated towards the quinoid ring through *pi*-electron delocalization or resonance. Hence; with the last two substituents, there is resonance effects opposing induction which is directed away from the ring. This explains why chloro and hydroxy groups are less electron attracting than the cyano group. Although oxygen is more electronegative than chlorine, there is more electron donation for functions containing oxygen. This is due to the difference in atomic size. Since oxygen is a smaller atom than chlorine, its *p*-orbitals which accommodate one of the lone pairs of electrons can overlap maximally with the *p*-orbital of carbon in the quinoid ring. Maximum overlap of orbitals is required for greater delocalization of electrons. The greater the delocalization, the greater is the resonance effects which counteracts induction in the case mentioned above. The result indicates that the greater the electron attracting effect (the more positive the substituent constant) the greater would be the energy gap between the LVMO and the HOMO and consequently the shorter would be the manifested wavelength in the ultraviolet spectrum. The perturbation of the energy levels can thus be reasoned out on the basis of substituent influence.

Employing the same quinone but changing the solvent system (Table 1) it is observed that there is a progressive increase in the wavelength of absorption with increasing dielectric constant of the solvent (or increasing polarity of the solvent). The more polar the solvent, the greater is the stabilization of the excited state than the ground state. The excited state exhibits more positive charge than the initial or ground state as a conse-

quence of electronic excitation (in this case from II to II*). The increment in the wavelength of absorption is very small due to the compensatory effect of the solvent for the perturbation effect due to the substituent. The energy gap between the HOMO and the LVMO in a nonpolar solvent should be larger than in a polar solvent. The polar solvent will stabilize the excited state better resulting in a smaller energy gap between the HOMO and the LVMO, thereby manifesting an absorption at a longer wavelength in the electronic spectrum. The extinction coefficients (ϵ) do not follow the trend as the wavelength as these measure the probability of exciting an electron from the HOMO to the LVMO [Jaffe and Orchin (1962) and Silverstein and Bassler (1965)].

The rate of reaction was determined for the different quinones in separate solvent systems (Table 3).

TABLE 3.—*Reaction rate with 9,10-dihydroanthracene.*

Quinone	Solvent	Reflux temp °C	λ-max	D	k_2 (1/mole-sec)* x 10^{-3}
chloranil	dioxane	103	285	2.209	2.64 ± 0.65
chloranil	chlorobenz.	130	287	5.62	6.93 ± 0.08
chloranil	n-amyl alc.	136	288	13.9	10.95 ± 0.97
chloranil	ethanol	74	288	25.0	16.72 ± 0.17
chlor. acid	dioxane	103	305	2.209	0.42 ± 0.05
DDQ	dioxane	103	282	2.209	18.91 ± 0.96

* Calculated using the method of least squares.

The choice of solvents in this study was dictated by considerations of polarity, related boiling points and capacity to dissolve reactants.

Using the same solvent (in this case dioxane), it was observed that the rate constants vary. The rate for DDQ is six to seven times faster than for chloranil, and, for chloranil six to seven times faster than for chloranilic acid. These differences can be attributed to substituent influence since the same medium was employed. The more electron attracting substituent makes the quinone more electrophilic and subsequently enhances the proton abstracting power of the quinone in the dehydrogenation process.

Considering the same quinone in different solvents, the rate constants increase with increasing polarity of the solvent. It has been accepted that the greater the value of the dielectric constant of the solvent, the higher would be its polarity [Kosower

(1967)]. It appears that doubling the dielectric constant doubles the rate constant. The effect on the rate constant cannot be explained by solvent effect alone, since temperature effect has to be considered also. Since the temperature of the reaction was limited by the boiling point of the solvent (at reflux temperature), we assumed that the reaction is pro-Arrhenius and the rate values could be extrapolated to one temperature scale around the range of most of the solvents. This assumption is reasonably valid since our reaction does not have any of the features (e.g. explosion, etc.) of anti-Arrhenius reactions. In ethanol, the most polar of the solvents used but the one with the lowest boiling point, if temperature effect were more critical than dielectric effect, the rate should be lower than that in dioxane. However, the reverse is observed, indicating that dielectric constant becomes the more critical factor than temperature effect in the reaction studied.

The influences of substituent and solvent strongly indicates that the reaction follows a polar reaction mechanism.

With the results obtained from the above experiments, dehydrogenation was employed in the conversion of the nonconjugated unsaturated fatty esters in lumbang oil [*Aleurites moluccana* (Linn.)] to the highly polyunsaturated conjugated fatty esters. The major components of the oil are indicated in Table 4.

TABLE 4*

Fatty acid	Per cent
linolenic	25.0
linoleic	39.0
oleic	26.0
saturated FA's	10.0

* Data supplied by Dr. G. Weismann of Hamburg, Germany; from gas chromatographic analysis of lumbang oil supplied by Nasipit Lumber Company, 1969.

The highest conversion realized was only 4 per cent. The polyunsaturation was found to decrease with time [Moore and Waters (1960) and O'Leary (1967)]. Theoretically, isomerization alone of linolenic would give 25 per cent of conjugated fatty trienic acids and dehydrogenation of linoleic would add to this about 40 per cent, not including products which could arise from oleic acid. The low conversion may be due to (*a*) polymerization accompanying the reaction say by the Diels-Alder reaction [Kirschenbauer (1967) and Kosower (1967)] and/or (*b*) presence of naturally occurring antioxidants in the raw oil which lessens the activity of the quinone.

ACKNOWLEDGMENT

The authors are grateful to the National Science Development Board for the graduate fellowship extended to Mr. Raul Obach, without which this work could not have been possible; to the Nasipit Lumber Company and the Philippine Wallboard Corporation for supplying us with a new laboratory at the University of the Philippines where this work was undertaken and the supplies needed for this study; to Dr. Clara Y. Lim-Sylianco for allowing us the use of her equipment and library; and to Dr. Mary Danguilan for her constructive criticisms.

REFERENCES

BAILEY, A. E. (1951). Industrial Oils and Fat Products. New York: Interscience Publishers, Inc., 967 pp.

BARNARD, J. R., and L. M. JACKMAN (1960). Hydrogen transfer. Part X. The dehydrogenation of hydroaromatic hydrocarbons by quinones. Jour. Chem. Soc. (London), p. 3110.

BRAUDE, E. A., L. M. JACKMAN, R. P. LINSTEAD, and G. LOWE (1960). Hydrogen transfer. Part XIII. The kinetics of dehydrogenation of 1, I, 1-isubstituted 1, 2-dihydronaphthalenes and tetralins by tetrachloro, 1, 2-benzoquinone. Jour. Chem. Soc. (London), p. 3133.

BRICK, B. A. *et al* (1952). Spectrometric method of polyunsaturated fatty acid determination. Jour. Am. Oil Chem. Soc. **29**: 279, and refrences cited therein.

BROWN R. F., and L. M. JACKMAN (1960). Hydrogen transfer. Part XV. The synthesis and cyclodehydrogenation of 2-diphenylmethylstyrene. Jour. Chem. Soc. (London), p. 3144.

CAVENAUGH, J. R., and B. P. DAILEY (1961). Proton chemical shifts for the alkyl derivatives. Jour. Chem. Phys. **34**: 1099.

CHARTON, M. (1964). Definition of inductive substituent constants. Jour. Org. Chem. **29**: 1222.

CREIGHTON, A. M., and L. M. JACKMAN (1960). Hydrogen transfer. Part XIV. The quinone cyclodehydrogenation of acids and alcohols. Jour. Chem. Soc. (London), p. 3138.

DAILEY, B. P., and J. N. SCHOOLERY (1955). The electron withdrawal power of substituent groups. Jour. Am. Chem. Soc. **77**: 3877.

HAMMETT, L. P. (1971). Physical Organic Chemistry. New York: McGraw Hill Book Co., Chaps. 3, 4, and 7.

HENDRICKSON, J. B., D. J. CRAM, and G. S. HAMMOND (1970). Organic Chemistry. New York: McGraw Hill Book Co., 745 pp.

HINE, J. (1962). Physical Organic Chemistry. New York: McGraw Hill Book Co., 84 pp.

HINZE, J., M. A. WHITEHEAD, and H. H. JAFFE (1963). Electronegativities. Jour. Am. Chem. Soc. **85**: 148.

HUHEEY, J. E. (1965). The electronegativity of groups. Jour. Phys. Chem. **69**: 3284,

JAFFE, H. H. (1953). A re-examination of the Hammett equation. Chem. Rev. **53**: 191.

JAFFE, H. H., and H. ORCHIN (1962). Theory and Application of Ultraviolet Spectroscopy. New York: John Wiley & Sons. Inc., 171 pp.

KIRSCHENBAUER, H. G. (1960). Fats and Oils. New York: Reinhold Pub. Corp., 27 pp.

KOSOWER, E. M. (1967). Introduction to Physical Organic Chemistry. New York: John Wiley & Sons, Inc., 207 pp.

MARCH, J. (1968). Advanced Organic Chemistry. New York: McGraw Hill Book Co., 158 pp.

MOORE, R. F., and A. W. WATERS (1953). Some photochemical reactions between quinones and hydrocarbons. Jour. Chem. Soc. (London), p. 3405.

MOORE, R. F., and A. W. WATERS (1960). Official and Tentative Methods of Analysis. 9th ed. Washington D.C.: Association of Official Agricultural Chemists (AOAC), 369 pp.

O'LEARY, W. M. (1967). The Chemistry and Metabolism of Microbial Lipids. New York: The World Pub. Co., 98 pp.

SILVERSTEIN, R. M., and G. C. BASSLER (1965). Spectrometric Identification of Organic Compounds. 2nd ed. New York: John Wiley & Sons, Inc., 20 pp.

ELECTRONIC AND STRUCTURAL EFFECTS ON RATES AND EQUILIBRIA, V

NUCLEOPHILIC REACTIVITY OF SOME ALIPHATIC AMINES*

By WENDEL Y. LIM,[1] BERNADETTE I. LAZARO, and FLORENCE MANLIGAS-NACINO
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Nucleophilicity of some aliphatic amines against 2, 4-dinitrochlorobenzene (DNCB) as the reference electrophile was measured in dioxane at 450 nm and 30°C. The observed second order rate constants (k_2, 10^{-4} liter/mole-sec) for the amines are: n-butylamine, 9.63; n-propylamine, 8.80; isobutylamine, 4.64; isopropylamine, 2.10; sec-butylamine, 1.54; and t-butylamine, 0.36. Results are interpreted in the light of current electronic and structural theories.

The incorporation of the concept of electron donating (or attracting) tendency of atoms and groups or functions into the explanation of certain physical and chemical properties and its use in the interpretation of electrical dissymetry of valence bonds in molecules, has been emphasized in the modern structural theory of organic chemistry [Ferguson (1958), Hendrickson *et al* (1970), Hine (1962), Ingold (1971), March (1968)]. In relation to this trend, we have conducted investigations [De Vera and Lim (1970), Lim and de Vera (1970)] on the properties of organic compounds which contain the amino ($-NH_2$) group and related these properties to structure. In one publication [Lim and de Vera (1970)], it was observed that molecular complexation and steric effects played very important roles in the nucleophilic reactivity of aromatic amines. The present investigation extends the study to some aliphatic amines.

EXPERIMENTAL PART

The N-alkyl 2,4-dinitrophenylaniline products were prepared using standard procedures [Lim and de Vera (1970)]. From the purified products, individual reference absorbance-concentration curves were obtained.

To 10 ml of 10^{-2}M 2,4-dinitrochlorobenzene (DNCB) in reagent grade dioxane in a 50-ml volumetric flask were added 10 ml of 10^{-2}M amine. The stoppered flask was vigorously shaken

* For previous paper in this series, *see* W. Lim, M. Luga-Ramiro, and N. de Vera, "Electronic and Structural Effects on Rates and Equilibria. IV. Conformational Influence on the Nucleophilicity of Cyclic and Alicylic Amines," Nat. Appl. Sci. Bull. **23** (1971), in press.

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and then placed in a constant temperature bath (30 ± 1°C). Aliquots were taken at definite time intervals and these were analyzed at 450 nm using a Beckman DU Spectrophotometer. Absorbance values were extrapolated to the concentration values with the aid of the reference curves. Rate constants (Table 1) were calculated [De Vera and Lim (1970)] using the graphical, numerical and the least squares methods.

RESULTS AND DISCUSSION

From Table 1 the second order rate constants for the amines are indicated. The normal or straight chain amines gave high values followed by branched chain amines in the order of branching complexity.

TABLE 1.—*Thermodynamic and rate constants of aliphatic amines used in this study.*

Amine (RNH_2) R	pKa[1]	pKa[2]	k_2[3] (10^{-2} 1/m-s)	k_2[4] (10^{-4} 1/m-s)
n-Pr	10.6	10.53	5.80	8.80 ± 0.01
i-Pr	10.6	10.63	0.60	2.10 ± 0.10
n-Bu	10.6	10.59	6.00	9.63 ± 0.30
i-Bu	10.4	10.43	4.10	4.64 ± 0.02
s-Bu	10.6	10.56	0.55	1.54 ± 0.05
t-Bu	10.4	10.45	0.02	0.36 ± 0.02

[1]Hall and Sprinkle (1932), [2]Hall (1657), [3]Brady and Cropper (1950), ethanol as solvents; [4]using dioxane as solvent, by method of least squares.

It has been shown by several investigators [Brown (1945), Brown and Pearsall (1945), Ferguson (1958), Taft (1953)] from the consideration of polar effects of alkyl groups that the base strength of aliphatic amines increases with the number of alkyl groups. Thus theoretically, a tertiary amine is more basic than a secondary amine which in turn is more basic than a primary amine. This general trend

$$R_3 N > R_2 NH > RNH_2$$

may not be followed where medium effects such as those observed by Brown [Brown (1945); Brown and Pearsall (1945) or steric effects exerted by some substituents [Hall (1957), Taft (1953)] become important. In this study, primary amines were chosen as model compounds. These primary amines differ only at the *alpha* (α) - carbon as shown below;

γ β α	α	CH_3
$CH_3CH_2CH_2NH_2$	$CH_3CH_2CH_2CH_2NH_2$	$CH_3CH\text{-}NH_2$
I	II	III
CH_3	CH_3	CH_3
$CH_3CH\text{-}CH_2NH_2$	$CH_3CH_2CH\text{-}NH_2$	$CH_3C\text{-}NH_2$
		CH_3
IV	V	VI

In compounds I, II, and IV (n-propylamine, n-butylamine and isobutylamine, respectively) the *alpha*-carbon is primary, whereas in isopropylamine, III and sec-butylamine, V, this center is secondary and in t-butylamine, VI, this center is tertiary.

On the basis of purely electron-donating capacity (polar effect) of alkyl groups, the order of basicity [Hall (1957)] and possibly reactivity [Hall (1957), Wells (1963)] should follow the order:

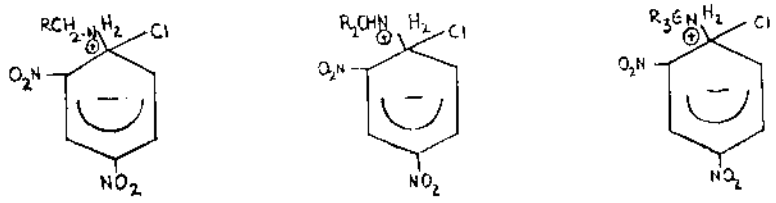
t-Bu > s-Bu > i-Pr	≃	i-Bu > n-Bu > n-Pr
-6* 0.68 0.77 0.79		0.79 0.85 0.86

The values indicated at the bottom of each function represents the magnitude of electron donation [Hall (1957)] in the form of substituent constant (6^*). The greater the electron-donating capacity of the alkyl group, the greater should be its effect in making the lone pair of electrons on nitrogen more available for coordination. From the pKa values indicated in Table 1, the amines exhibit almost the same basicity. A difference in terms of 0.1 power of 10 or two units in the linear scale of basicity, does not reflect significantly in the relative basicity of these compounds. The results therefore cannot be explained in terms of amine basicity. On the basis of pure polar effects, the sequence indicated above should be the order followed. Neither of these two schemes was observed. The sequence obtained from the values indicated in the last column of Table 1, is:

$$\text{n-Bu} > \text{n-Pr} > \text{i-Bu} > \text{s-Bu} > \text{t-Bu}$$

The sequence due to steric effects is just the opposite of this observed order [Wells (1963)]. It is evident that steric order is followed by the amines used in this study. This order is, however, affected by some polar contribution as in the case of n-Bu and n-Pr.

The steric influence can be easily understood if one examines the transition states and intermediates which asise from the interaction of the electrophile, DNCB, and the corresponding amine (see structures VII, VIII, and IX). Examination of Dreiding models which simulate the transition state [Hammond (1955)] or Mesenheimer intermediate, showed



VII VIII IX

that substitution at the *alpha*-carbon of the amine exerts some steric interaction with the *ortho*-nitro group of DNCB. The more sterically hindered this position became, the more pronounced was the observable effect on the rate of reaction. This is so because a steric effect at the transition state [Frost and Pearson (1961)] would destabilize the activated state, requiring a higher energy of activation and thus a slower rate of reaction. This can be indicated by an energy profile, as in Figure 1.

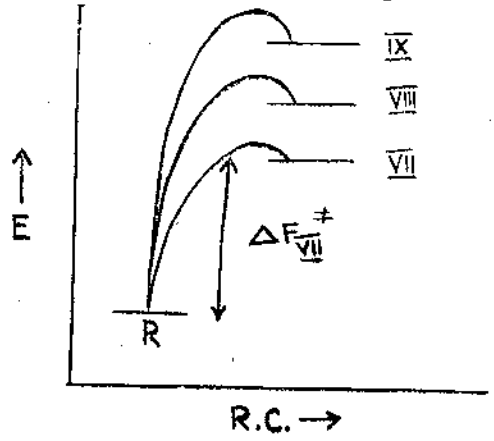


FIG. 1. Energy profile for the three transition states or intermediates.

Our results therefore are best interpreted in terms of steric hindrance at the transition state or intermediate. Any destabilization at these stages during the reaction would increase the potential energy of these states thereby causing a slower rate of reaction. In this study, it has been shown that steric effects of the type

classified by Brown [Brown (1945), Brown and Pearsall (1945)] as F-strain was found to overcome polar and thermodynamic effects.

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REFERENCES

BROWN, H. C. (1945). Studies in stereochemistry. VII. The effect of F-strain on the relative base strength of ammonia and ethylamine. Jour. Am. Chem. Soc. **67**: 1452.

BROWN, H. C., and H. PEARSALL (1945). Studies in stereochemistry. VIII. The effect of F-strain on the relative base strength of isopropyl and t-butylamines. Jour. Am. Chem. Soc. **67**: 1765.

BRADY, L., and F. R. CROPPER (1950). The reaction between amines and 1-X-2,4-Dinitrobenzenes. Jour. Chem. Soc. (London), p. 507.

DE VERA N., and W. Y. LIM (1970). Molecular complexes. I. Nat. Appl. Sci. Bull. **22**: 12.

FERGUSON, L. (1958). Modern Structural Theory of Organic Chemistry. New York: John Wiley and Sons, Inc., 58 pp.

FROST, A. A., and R. G. PEARSON (1961). Kinetics and Mechanisms. 2nd ed. New York: John Wiley and Sons, Inc., 78 pp.

HALL, H. K. (1957). Correlation of base strength of amines. Jour. Am. Chem. Soc. **79**: 5441.

HALL, N. F., and M. R. SPRINKLE (1932). Relations between the structure and strength of certain organic bases in aqueous solution. Jour. Am. Chem. Soc. **54**: 3469.

HAMMOND, G. S. (1955). A correlation of reaction rates. Jour. Am. Chem. Soc. **77**: 334.

HENDRICKSON, H., D. J. GRAM, and G. S. HAMMOND (1970). Organic Chemistry. New York: McGraw-Hill Book. Co., 319 pp.

HINE, J. (1962). Physical Organic Chemistry. 2nd ed. New York: McGraw-Hill Book Co., 60 pp.

INGOLD, C. K. (1971). Structure and Mechanism in Organic Chemistry. London: Oxford University Press, 20 pp.

LIM, W. Y., and N. DE VERA (1970). Polar and steric effects on rates and equilibria.I. Nucleophilicity of some aromatic amines. Nat. Appl. Sci. Bull. **22**: 26.

MARCH, J. (1968). Advanced Organic Chemistry. New York: McGraw-Hill Book Co., 19 pp.

TAFT, R. W. (1953). The general nature of the propotionality of polar effect of substituent groups in organic chemistry. Jour. Am. Chem. Soc. **75**: 4231.

WELLS, P. R. (1963). Linear free energy relationship. Chem. Rev. **63**: 171.

ADVERSE REACTIONS TO LYSERGIC ACID DIETHYLAMIDE IN ANIMALS: NEST-BUILDING AND GENERAL MATERNAL CARE IN RATS

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THREE TEXT FIGURES

ABSTRACT

Multidisciplinary research has shown that untoward reactions to LSD include chromosomal aberrations and disruption of adaptive response patterns in man and animals.

Nest-building in rats forms two types of adaptive patterns. Among normal male and female rats nest-building basically has a thermoregulatory function. In the postpartum lactating female rat, nest-building is critically determined by endocrinological state although temparature may be an additional factor.

Five dose levels of LSD and a control saline dose were injected intraperitoneally into 40 primiparous rats. The relationship between dosage in logarithmic units and mean reduction in paper collection for nest-building was essentially linear, suggesting that the method may be of use in biological assay. Other aspects of Physiology, maternal care and viability of young were also adversely affected by the drug.

The results of this and many other investigations show that LSD is strongly detrimental to the well being of animals and interferes with the adjustments of these organisms to their surroundings.

INTRODUCTION

Much information has accumulated in recent years regarding the adverse effects of lysergic acid diethylamide (LSD-25). Reports include such findings as: *in vitro* and *in vivo* chromosonal aberrations in man [Cohen (1968) and Cohen *et al* (1967)] and nonfunctional web-spinning among spiders [Witt (1951)].

The present study describes the effect of LSD-25 on nest-building an important aspect of maternal behavior in rats [Kinder (1927), Ray (1956), and Rosenblatt and Lehrman (1963)]. Other maternal changes due to this drug are presented. Findings of other workers are also related to the data of the present experiment to show the general disruptive action of LSD-25 upon the dynamic equilibrium of organisms and the subsequent deterioration of their adaptive responses to the environment.

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MATERIALS AND METHODS

Subjects and apparatus.—Thirty primiparous female rats of the G-4 strain (Jackson Laboratory) were used as subjects. The apparatus, adapted from that of Richter (1956), consisted of four wooden boxes, 2 feet long by 2 feet wide by 18 inches high, provided with metal trays to facilitate cleaning. On the outside of each box a roll of heavy-duty toilet paper was mounted with the end of the roll fed through a narrow slot into the box. The rolls were raised to permit paper to be stripped off without undue friction (Fig. 1).

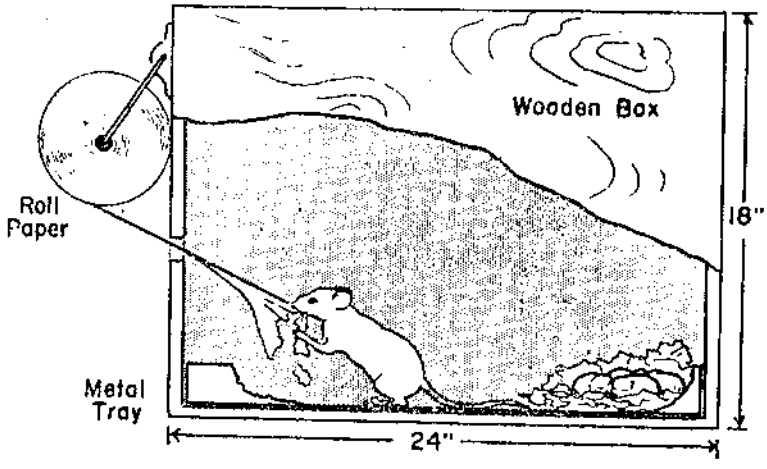

FIG. 1. Experimental box.

Procedure.—Pregnant female rats were removed from males and isolated until the day of parturition. On this day each female and her "pups" were moved into an experimental box. Water, food pellets, and nest-building material were made available. One day was allowed for the subject to become accustomed to the box before testing began.

During test sessions the rate of paper collection was measured by weighing the paper roll and subtracting from the original weight. Measurements were made for 6 hours (divided into three 2-hour periods) before and 6 hours (divided into three 2-hour periods) following injection of LSD-25. The mean of the three periods of nest-building prior to injection for each animal was used as the base line figure for that animal. The base line figure was divided into the amount of nest material used by the rat within 2 hours after injection, resulting in a figure which

would represent the percentage of the original base line that occurred under the effect of LSD. The figure representing nest material used by the rat under drug conditions was substracted from the base line figure to obtain an estimate of gross difference. Since the rats varied in base line amounts, this last figure is not completely representational although it happened to yield similar results as the percentage figure in the data. The effects of the drug at each post-treatment interval were measured in terms of percentage change from the average of three pretreatment observations. In addition observations were made as to whether the paper removed was used to construct a nest.

Other studies, of physiological and behavioral type, were not employed here, since it was important to control for factors which might have disturbed the test situation. In the course of the experiment it was possible to make a few notes that were related to general aspects of maternal care.

All doses of LSD-25 (DELYSID) were administered subcutaneously. Calculated dosages in mgm/kgm gross body weight were 0.416, 0.208, 0.104, 0.052. 0.026, and 0.0 (physiological saline). Twenty-five subjects received one dose level only and were used for only one test. The remaining five rats were tested in the four lower dose levels to ascertain if individual differences in drug reaction were more influencial than the effect of the drug. In this situation each rat was tested every other day, given one day of rest.

In the course of the experiment an additional 10 rats were studied, consisting of four animals given 0.026 mgm/kgm and six given 0.208 mgm/kgm subcutaneously. These results are not included in Figs. 2 and 3 since noise and other potentially disturbing factors in the environment could not be controlled. On two occasions (seven rats) trials were interrupted at about five and twenty minutes after conclusion of the first 2-hour postdrug period. On another occasion (three rats) disturbance occurred 6 minutes before expiration of the first period. Observations on the experiments were therefore terminated at the times the workmen intruded.

RESULTS

No significant differences were found between the results on the five animals given repeated doses and those receiving a single dose. Therefore, data from both groups have been combined in Figs. 2 and 3. The schedule in this study of alternating

injection and rest days may account for the lack of tolerance to LSD in contrast to the results of Freedman *et al* (1958 and 1964) who injected rats on a daily basis. However, other rat studies [Hofer and Osmond (1967)] report no tolerance to LSD. According to the analysis of variance [Snedecor (1956)] in Table 1 the amount of numerical variance due to individual variability did not statistically eliminate the still greater difference due to treatment on the amount of nest material used by the five animals in the factorial design.

TABLE 1.—*Analysis of variance.* The effect of four dose levels of LSD on the same rats (N = 5).*

Paper collection: Percentage of original base line

Source	d.f.	Sum of squares	Mean square	F Ratio	P
Treatments	3	17,354.2	5,784.73	12.88	< .001
Individuals	4	11,815.8	2,953.95	6.58	< .01
Residual	12	4,491.8	449.18		
Total	19	33,661.8			

Paper collection: Grams difference from original base line

Source	d.f.	Sum of squares	Mean square	F Ratio	P
Treatments	3	5,341.3	1,780.4	18.34	< .001
Individuals	4	2,191.6	547.9	5.64	< .01
Residual	12	1,165.6	97.1		
Total	19	8,698.5			

* According to Snedecor (1956).

Fig. 2 shows the mean percentage reduction in amount of paper used during the first 2-hour postinjection period. The significance of differences between adjacent dose levels was estimated by the Mann-Whitney U-test [Siegel (1956)]. All differences between adjacent levels were significant ($P > .05$) except that between 0.052 and 0.026 and that between 0.416 and 0.208 mgm/kgm. The number of cases in the latter comparison was, of course, too small for any significance to be established, in a statistical way. The percentage reduction in the amount of nest material used was directly proportional to the logarithm of the LSD dose, individual variation being insufficient to dsturb this relationship.

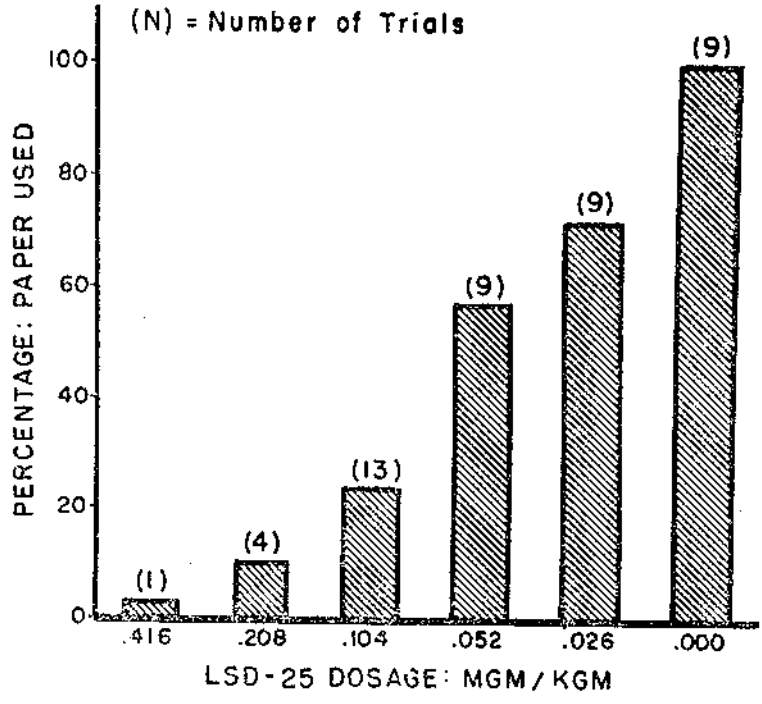

FIG. 2. Percentage reduction in paper collection.

The amount of paper collected returned to base level within 6 hours in subjects receiving 0.052 mgm/kgm or less, but animals given larger doses still showed some depression at the end of the 6-hour period.

At dose levels up to 0.052 mgm/kgm paper collection was reduced but most animals constructed nests. At 0.104 mgm/kgm and above paper was collected by some subjects who did not use it to build nests (Fig. 3). In general, nest-building was disrupted more in subjects whose paper collection was most reduced, but the two measures were not perfectly correlated.

Animals receiving 0.052 or 0.104 mgm/kgm sometimes built nests and sometimes did not. The mean reduction in paper collection for nest-builders was 48 per cent (range 11 to 81 per cent) and for non-nest-builders 77 per cent (range 25 to 93 per cent). Although the difference was significant there was marked overlap between the groups. Paper collecting and nest-building seem to be differently sensitive to the drug. The dose-response relationship for actual nest construction is far from linear and approaches a threshold effect between 0.052 and 0.026 mgm/kgm.

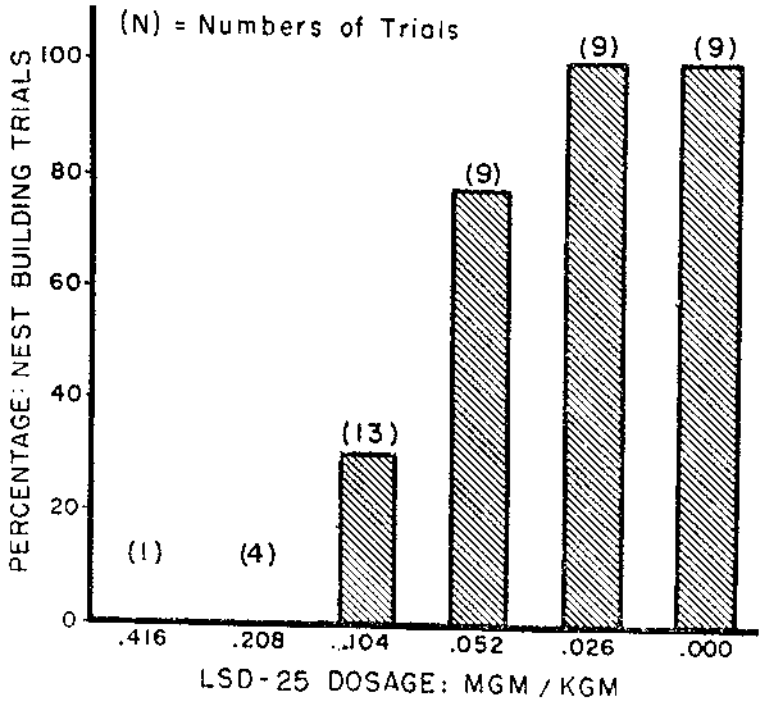

FIG. 3. Percentage of nests built.

The orderly patterning of acts necessary for nest-building was not always disrupted by low doses of LSD which significantly decreased the amount of paper collected; however, paper collecting continued at a low rate with higher doses that abolished nest construction. Although paper collection is a more sensitive indicator of LSD-25 effects, it is resistant to high doses.

When the paper roll was weighed at 4 and 6 hours after injection of LSD, the rats in all dose levels showed an increase in paper collection. In the case of 0.025 and 0.062 mgm/kgm the amount of nest material used was at base level by 6 hours after injection. For the groups given 0.208 and 0.102 mgm/kgm the amount of nest material used was at 50 per cent base level by 6 hours after injection. The one case of 0.416 mgm/kgm used 20 per cent of base level amount after injection of LSD. This is evidence that there is a relationship between duration of effect of LSD and amount of LSD administered. The author was unable to carry the measurements out to any longer periods of time.

Data from additional experiment.—The mean percentage reduction from baseline in paper collection was 32 per cent for the 0.026 mgm/kgm group and 89 per cent for the 0.208 mgm/kgm

group. Although the results for the interrupted observations are not significantly different from those in Figs. 2 and 3, at the same dose levels, there is a possibility of some effect at the 0.026 mgm/kgm level. These data are included therefore as supportive evidence only.

Other observations.—During the immediate postinjection period LSD-rats were excitable and over-active, much more so than those receiving the saline placebo who calmed down quickly.

All control subjects wound the crumpled paper into well-formed conical nests but some treated subjects left the paper in loose folds even when it was placed on the nest site. The saline controls built their nests in one spot, either in corners or the rear of the box, and used this as the site of subsequent nests. Drugged rats altered nest locations and one mother (at 0.102 mgm/kgm) built three small nests during one session. No nests were built at dose levels of 0.208 and 0.416 mgm/kgm.

Small amounts of urine were seen on the floors of control boxes. This was not observed among any of the experimental rats. Measurements of urine and feces were not considered practical in this study.

In experimental boxes of rats receiving the two high doses (with one exceptional at 0.208 mgm/kgm) the young were observed widely scattered around the floor of the box. Among these and also some lower dose rats, the mothers were observed wandering around inside the box away from the young or original nest site. Retrieving and cleaning of young appeared to be reduced at low as well as high drug levels. These mothers engaged in considerable self-licking, a grooming pattern that did not appear in the saline controls.

Several experimental factors could have affected mortality ratio of the offspring during the 24 hours following the end of the second 6-hour period so these data were not given detailed analysis. In brief, mortality of young was 70 per cent for the two higher doses (0.208 and 0.416 mgm/kgm), 25 per cent for rats given only one of the three lower LSD injections, 8 per cent for the saline controls, and 40 per cent for the five rats given the saline and the three lowest LSD doses. Mortality figures for the latter group are, of course, not directly comparable since they are based on deaths occurring during the several days their mothers were tested. This indicates that there was considerable variation to the experimental stress factors among the "pups."

One rat, from the interrupted study, injected with 0.208 mgm/kgm, was taken out of the box. It showed pilo-erection, and its head shook violently when touched.

DISCUSSION

Nest-building and maternal care of rats.—The present experiment was designed to study the effects of LSD-25 on a behavioral pattern in mammals which could be measured quantitatively and which required some temporal patterning of responses. Nest-building in the lactating female rat appeared to meet these conditions. Although no pattern of behavior should be considered as independent of learning, it appears that nest-building, in primiparous rats is more critically determined by endocrinological state and by environmental stimuli than by prior experience [Kinder (1927), Richter (1922 and 1956)].

Two types of nest-building occur among laboratory rats. The first type takes place in both nonpregnant female and in normal male rats [Jones *et al* (1953), Kinder (1927), McQueen-Williams (1935), Richter (1922, 1937, 1956), Rosenblatt and Lehrman (1963), Shirley (1928), Sturman-Hulbe and Stone (1929), Wiesner and Sheard (1933)]. It appears at 20 days of age [Kinder (1927)] and reaches its maximum between 175 [Richter (1922)] and 210 [Shirley (1928)] days of age depending upon the strain of rats and laboratory conditions. No sexual difference is seen in nest-building until the time of puberty. Nest-building is higher in female rats before puberty than after puberty until pregnancy.

The nest-building of rats contributes to the thermal regulation of the organism. Influence of temperature is most clearly seen in male rats [Kinder (1927)]. In the adult female the responses to temperature are complicated by the cyclic oestral nest-building variations [Kinder (1927)]. Vaginal smear records show a relationship between rhythmical variations in nest-building and the 5-day oestral cycle of rats. There was little nest-building when cornified cells appeared in vaginal smears [Kinder (1929)]. The body temperature of rats rises following ovulation [Richter (1956)]. Low environmental temperatures result in increased nest-building. At temperatures above 80°F the nest-building drops out for all animals except mothers suckling young [Kinder (1927)].

Increase of nest-building among normal rats is accompanied by increase in food intake suggesting that these two responses

have a similar physiological significance [Kinder (1927)]. A constant inverse relationship exists between food intake and running activity and between activity and nest-building in normal rats [Richter (1956)]. Hoarding of food and nest-building are, however, quite distinct forms of behavior and not entirely interdependent [Munn (1950)].

The homeostatic significance of nest-building in nonlactating rats is shown by experiments involving removal of pituitary and thyroid glands. Thyroidectomy of male rats leads to enlarged pituitaries plus development and appearance of maternal behavior such as retrieving and cleaning [McQueen-Williams (1935), Richter (1937 and 1956)]. This operation lowers the rate of metabolism, thus reducing heat production and leading to increase nest-building [Richter (1956)]. Removal of the pituitary gland causes a reduction in body temperature, weight, and degenaration of the thyroid, which results in an impaired ability to increase metabolism at low temperatures, thus resulting in more nest-building [Richter (1956)]. Thyroxine given to hypophysectomized rats prevents the effect of the operation on nest-building. [Richter (1956)]. Nest-building represents a thermoregulatory response in the normal rat with changes in external temperature and in thyroidectomized and hypophysectomized rats with changes in metabolism [Richter (1956)].

Pregnancy and parturition alter the factors regarding nest-building of female rats [Richter (1956)]. In the case of pregnant rats, nest-building may contribute nothing to their own thermoregulation, and so is not homeostatic in this sense. This illustrates that fixed action patterns are diverse both in their functions and their causes [Barnett (1963)].

Maternal behavior of rats is congenital, appearing even in rats isolated at birth and receiving no social contact except mating. It is as evident in primiparous rats as in those that have delivered several litters. Important aspects of maternal behavior include: activities at parturition, nest-building, retrieving, cleaning, and nursing of young [Munn (1950). Richter (1956), Rosenblatt and Lehrman (1963), Sturman-Hulbe and Stone (1929), Wiesner and Sheard (1933)]. This discussion will center on nest-building, since it is emphasized in the present experiment.

After conception, female rats increase nest-building to levels much higher than normal rats [Richter (1956)]. The greatest increase takes place on the day of birth despite wide ranges of

environmental temperatures. Nest-building usually continues at a high level for 7 days (in viable litters) and then drops sharply until the ninth day postpartum where it is maintained at the prebirth level. Nest-building is very persistent during the 7-day postpartum period even at temperatures above 80°F [Kinder (1927), Richter (1956)].

Other research workers [Rosenblatt and Lehrman (1963)] find increased nest-building 3 to 4 days before parturition. The delivery nests are low with no walls and shaped like mats. The postpartum nests are built in corners or back portions of cages where air circulation is at a minimum. They are high, compact, often conical piles, enclosing a cave with a tunnel next to one wall, and are so well made that they can be picked up intact with one finger [Kinder (1927), Richter (1956), Rosenblatt and Lehrman (1963)].

During the first week the pups are retrieved, cleaned and nursed by their mother. The pattern of maternal care changes by the end of the second week. As their eyes open the young become mobile and more independent of the mother and the nest becomes lower and wider [Rosenblatt and Lehrman (1963)].

Sturman-Hulbe and Stone (1929) believe that maternal behavior in the rat is activated by factors arising within the parturient female rather than stimuli in the external environment. Evidently the destruction of mammary glands does not abolish or destroy maternal behavior hence attention to the young is not based on the need to have milk withdrawn [Munn (1950)].

More recent data [Rosenblatt and Lehrman (1963)] support a multifactorial explanation. Other behavioral changes, such as alterations in grooming patterns, occur in bravid rats. Self-licking is pronounced during pregnancy and shows a great decrease at parturition after which the cleaning of the young predominates, with a shift in the mother's orientation from her own body to that of the newly born young.

Parturition represents a physiological shift from dominance of the placental hormones to that of the pituitary gland, which is more subject to neural (stimulus) control. The young provide sources of stimulation capable of inducing pituitary activity and lactation. This stimulation helps to maintain the maternal condition [Rosenblatt and Lehrman (1963)]. Changes in nest-building behavior are influenced by the reproductive state of the mother, evidently through action of hormones on the central

nervous system [Wiesner and Sheard (1933)]. Feedback phenomena occur in nursing where lactogen (milk-stimulating hormone) shows pituitary action on the mammary tissue and in turn oxytocin production in the posterior pituitary is stimulated during the period of milk production by sucking of the infant at the nipple.

Temperature cannot be entirely discounted in studies of maternal nest-building. Gelineo and Gelineo (1952) find that parturient rats, if given a choice, make their nest at low temperatures which favor the growth of the young. Richter (1956) surmised that in the lactating rat heat is lost in keeping the babies warm and in manufacturing and supplying milk. More heat is produced by increasing the food intake and conserved by increasing the amount of paper used in covering the body. Inactivity helps to conserve energy and in heating the body.

The altered pattern of nest-building and general maternal care resulting from LSD may have some thermal involvement, for although the drug increased rat temperature in toxic doses it caused hypothermia in low doses [Rothlin (1957, 1957a, 1957b)]. This contrasts with the general pyrogenic effect of LSD found among other species [Rothlin (1958, 1957a, 1957b)].

In this investigation the relationship betwten LSD dose level (in logarithmic units) and percentage reduction in paper collection is essentially linear. Generally monotonic (linear) regressions between LSD dosage and response were found by other workers in rats [Appel and Freedman (1965), Cook and Weidley (1957), Mahler and Humoller (1959), Ray and Bivens (1966), Uyeno (1966)]. Swiss mice [Wooley (1955)], cats [Furster and Vogt (1956)], squirrel monkeys [Uyeno *et al* (1968)], toads [Burgers *et al* (1958)], and man [Abramson *et al* (1955), Hofer and Osmond (1967)]. Relationships were linear after logarithmic transformation in other studies involving rats [Uyeno (1966), Uyeno and Mitoma (1969), Winter and Flataker (1956, 1957)], mice [Uyeno (1966), Uyeno and Benson (1965)], and pigeons [Berryman *et al* (1962)].

In the present study there was no evidence of an effect relating to Wilder's Law (The Law of Initial Values) [Lacey (1956)], as had been noted in some human studies involving LSD [Hofer and Osmond (1967)].

Since the relationship between dosage in logarithmic units and mean reduction in paper collection was essentially linear over

the range of LSD doses studied, the method may have some value for biological assay.

General topics.—Lysergic acid diethylamide, in addition to its well known physiological properties in man and animals, has been shown to disrupt learned behavior patterns in rats. Winter and Flataker (1956-1957) found that LSD decreased rope climbing in rats trained to avoid shock by means of this response. Cook and Weidley (1957) demonstrated that doses of 1.5 mgm/kgm blocked conditioned responses in trained rats.

This experiment has shown that two aspects of nest-building are unequally affected by LSD-25. The results are somewhat different from those cited above and those of Witt's (1951-1952) on the modifying (rather than eliminating) effect of this drug on web spinning in spiders. Webs constructed under the influence of LSD-25 were geometrically more uniform than ordinary webs, but less adapted to the specific place in which they were built. The increased geometrical uniformity of the webs was attributed by him to a reduction in the environmental control of spinning behavior so that an intrinsic control system took over and responses were run off in a stereotyped fashion. Rats given LSD do not not proceed to build nests with greater exactitude, but rather fail to carry out the more highly integrated activities of nest construction, while continuing, although at a reduced rate, the less complex behavior of paper collecting. These effects of LSD-25 are better explained by selective disruption of more complex integrative systems, than by a shift from extrinsic to intrinsic mechanisms of control.

Some detrimental effects of LSD on rats and other animals are summarized in Tables 2-6; and illustrate the basic anti-instinctual properties of the drug. Despite individual variations in initial reactions, tolerance and prolonged sequelæ, the major pattern resulting from LSD effect is *cacobiosis* (an abnormal way of life). The organisms adaptive mechanisms are disrupted, resulting in breakdown of the maintenance of dynamic equilibrium between it and the environment.

The marked effects of LSD upon nest-building and general maternal care of rats illustrate the possibility of added hazards of drug taking, in the immediate postpartum period, especially for nursing mothers. An article in the British Medical Journal [Editor (1967)] describes the risks of drug prescribing in pregnancy in which both the parent and the foetus are susceptible to

TABLE 2.—*Summary of some adverse effects of LSD-25 on rats (phylum Chordata, class Mammalia, order Rodentia family Muridæ).*

Source	Strain[2]	Dose level mg/kg	Method[3]	Effects
Sherman and Dickerson (1956)	n.s.	1.0	n.s.	Changes in body temperature (dependant on colonic "critical" ambient temperatures)
Alexander et al (1967)	n.s.	0.005	s.	Abortions, stillbirths, small litters, stunted offsprings
Brimblecombe (1963)	Wistar	0.5	i.p.	Defaecation (dependant on other factors)
Cook and Weidley (1957)	n.s.	1.5	s.	Blockage of pole climbing response, diarrhea, hypersensitivity, frequent urination
Freedman et al (1958)	Sherman	0.13	i.p.	Piloerection, bradycardia, pyrexia, mydriasis, respiratory arrest, confusion, nonresponsiveness to approach, slow rope climbing
Appel and Freedman (1965), Freedman et al (1964)	Sprague	0.13	i.p.	Decreased lever pressing for food reward
Cillut (1960)	Sprague-Dawley	0.13	i.p.	Reduced number of copulations preceding ejaculation in males, failure to copulate
Hamilton (1960)	Sprague-Dawley	0.5	i.p.	Increase in avoidance behavior, hypersalivation
Liberson et al (1961)	n.s.	1.0	n.s.	Blockage of goal directed behavior
Maffi and Constantini (1961)	n.s.	0.5	o	Blockage of timing of lever pressing response
Mahler and Rumpler (1959)	n.s.	0.3	i.p.	Increased delay in pole climbing, hypersalivation, peripheral vasodilation as evidenced by red color ears and paws, increased frequency of defaecation and urination
Marrazzi (1962), Ray and Marrazzi (1961)	n.s.	0.1	i.p.	Delay in lever pressing for water reward
Ray and Marrazzi (1961)	n.s.	0.08	n.s.	Delay in lever pressing for water reward
Ray and Rivers (1966)	Holtzman	0.05	i.p.	Decreased lever pressing for reward
Sackler et al (1966)	Wistar	0.5	s.	Increased adrenal weights; decreases in total white blood cell counts, in lymphocyte and eosinophil frequencies and in food consumption, reduction in thymus, thyroid and uterine weights
Uyeno (1966)	Wistar	0.001	i.p.	Inhibition of aggressive behavior and dominance
Uyeno (1968)	Long-Evans	0.0015	i.p.	Increase in starting latency in underwater swimming test, slower swimming time
Uyeno and Kitoh (1969)	Wistar	0.003	i.p.	Increased errors in underwater maze
Weltman and Sackler (1966)	Wistar	0.5	s.	Lowered rate of metabolism, decreased gonadal activity, increased adrenal weights, increased output of adrenocorticosteroids, decrease in food consumption and in genital and other organ weights
Winter and Flataker (1956-57)	Holtzman	0.175	i.p.	Impaired and slowed rope climbing performance, initial hyperactivity with later hypoactivity, confusion, violent head and body shaking, profuse salivation, refusal to eat, postural and movement problems, withdrawal to rear of cage
This study	C-4	0.026	s.	Decreased nest-building of postpartum females In larger doses: Changes in location of nest, nest material scattered, persistence of self-licking grooming pattern, increased mortality of offspring, "pups" abandoned, head shaking

[1] The taxonomic designations are according to: Evans (1899), Romer (1966), Simpson (1945). The taxons are not entirely comparable since in some orders (e.g. *Rodentia*) there are only laboratory forms, while others, such as *Primates*, consist solely of wild species.

[2] n.s. = not stated.

[3] Methods of drug administration
i.p. = Intraperitoneal; o. = oral; s. = subcutaneous

TABLE 3.—*Summary of some advarse effects of LSD-25 on mice (order Rodontia, family Muridæ).*

Source	Strain	Effect
Wooley (1955)	Swiss albino	Alteration of posture, walking backwards, marked tremors, agitation, shaking of head and whole body, reddening of ears, piloerection
Auerback and Rugowski (1957)	BALB/Cau C57BL6/Au C3H/HeAu F_1(BALB) X C57BL	Production of malformations in 37 per cent of embryos
Haley and Rutschmann (1957)	"white"	Hyperexcitability, increased sensitivity to touch and sound, peculiar muscle twitch in the lumbar area accompanied by alternate stamping of all four feet
Keller and Umbreit (1956)	n.s.	Violent head shaking when back of head was touched
Rothlin and Cerletti (1952)	Hereditary "waltzing"	Repression of waltzing movement, increase in excitation and spontaneous activity
Cohan (1968), Chakkabark *et al* (1968)	n.s.	Chromosomal aberrations (breaks, gaps, fragments) in gametes of males (meiotic damage)
Uyeno (1966), Uyeno and Benson (1966)	Swiss-Webster	Inhibition of attack behavior in males and females, piloerection, tachypnea, tremors, hypoactivity, withdrawal to rear of cage
Haley (1957)	n.s.	Straubé tail phenomenon, hyperexcitability and over-aggressiveness followed by stuporous condition lasting 12 hours

TABLE 4.—*Summary of some adverse effects of LSD-25 on other mammals.*

Taxonomy[1]	Source	Common[2] name	Effect
O. Rodentia F. Cricetidæ	Geber (1967)	hamster	Fetal malformations of the brain, spinal cord, liver and other viscera; body edema; localized hemorrhages; small fetuses; increased fetal mortality
O. Lagomorpha F. Leporidæ	Gogarty and Hills (1956)	rabbit	Pyrexia, hyperventilation, hyperexcitability
	Morita and Dille (1954)	rabbit	Rise in body temperature, decrease in skin temperature, hyperpnea, (pyrexia in cats and dogs)
	Ehazan and Sulman (1967)	rabbit	EEG effects
	McGaugh *et al* (1963)	rabbit	Blockage of discriminatory and instrumental responses, impaired movements, backward locomotion, continuous shaking of head, retreat to rear of cage, immobility, complete disorientation, decreased locomotion, ataxia
	Rothlin *et al* (1956)	rabbit	Hyperthermia, hyperglycemia, mydiasis, piloerection, cardiac acceleration
O. Carnivora F. Canidæ	Haley (1956) Haley and McCormick (1956)	dog	Whining, shaking of head, licking of chops, salivation, ataxia, retching, nausea, emesis, micturition, tachypnea, mydiasis with reactive pupils, continuous barking, "reversion from an adult behavior pattern to a puppy one"
	Dusraine and Frougis (1955)	dog	EEG, changes, hypoglycaemia, hyperbilirubinaemia, modified phosphate reactions in brain, degenerative changes in parenchyma of liver and kidneys
O. Carnivora F. Felidæ	Evarts (1956)	cat	Apparent temporary blindness, markedly reduced post synaptic response in the lateral geniculate nucleus to stimulus of the optic nerve
	Fey (1961)	cat	Blockage of conditioned auditory discrimination response
	Fey and Bradley (1958)	cat	Overresponsiveness to sensory stimulation, effect on unconditioned arousal response
	Elgar and Dille (1962), Forster and Vogt (1956), Gogarty and Dille (1956)	cat	"Rage" reaction, salivation, mydiasis, piloerection, hyperexcitability
O. Proboscidea F. Elephantidæ	West *et al* (1962)	Asian elephant *Elephas maximus indicus*	Death after being given a very small dose on a weight basis

[1] O = Order, F = Family

[2] No species or strain name stated except in case of elephant [West *et al* (1962)].

TABLE 5.—*Summary of some adverse effects on LSD-25 on primates.*

TABLE 5.- Summary of some adverse effects of LSD-25 on primates.

Taxonomy[1]	Source	Animals: Common name	Species name	Effect
F. Cebidae	Dyeno *et al* (1962)	squirrel monkey	*Saimiri sciureus*	Disruption of learned visual size discrimination performance
F. Cercopithecidae	Chen and Weston (1960)	rhesus monkey	*Macaca mulatta*	Loss of biting responses; tameness; catalepsy, ataxia, stupor, unable to grip cage
	Clark *et al* (1962)	rhesus monkey	*Macaca mulatta*	Deterioration of lever positioning response to avoid electrical shock
	Forster (1957)	rhesus monkey	*Macaca mulatta*	Impairment of visual acuity, lengthening of reaction time
	Evarts (1956)	monkey	*Macaca mulatta*	Loss of adaptive aggressiveness; tameness; prone (froglike) position; ataxia; temporary blindness insensitivity to painful stimulus
	Morita and Chorover (1958)	macaque monkey	n.s.	Decrements in accuracy in a "delayed alteration task" at low doses, rate of performance depressed at high doses
F. Pongidae	Baldwin *et al* (1959)	Chimpanzee	n.s. (Pan troglodytes ?)	Mydriasis; salivation; grimacing; hyperventilation; piloerection; beating at air; violent movements; crawling on back; ataxia

[1] F = Family

TABLE 6.—*Summary of some adverse effects of LSD-25 on other animals.*

Taxonomy[1]	Source	Animals: Common name	Species name	Effect
P. Platyhelminthes C. Trematoda O. Digenea F. Fasciolidae	Mansour (1956-57)	liver fluke of sheep	*Fasciola hepatica*	Alteration of rhythmic activity of fluke (in nerve, muscle preparation and intact parasite); increase in production of lactic acid
F. Mollusca C. Gastropoda O. Prosobranchiata F. Ampullaridae	Abramson and cf Jarvik (1955)	"mystery snail"	*Ampullaria cuprea*	Inhibition of muscle tone; influences snail to open its operculum, extrudine its tentacles, proboscis and gastropod; abnormal, wild muscular movements of gastropod which prevent snail from adhering to any surface; snail usually dies after 36 hours
P. Arthropoda C. Arachnida O. Araneida F. Argiopidae	Witt (1951-52)	spider	*Zilla Xiphosida, Meta reticulata, Araneus diadema*	Nonadaptive pattern of web spinning; reduction in catching area of web
P. Chordata C. Osteichthyes O. Atheriniformes F. Cyprinodontidae	Keller and Umbreit (1956a)	guppy or rainbow fish	*Lebistes reticulatus*	Distortions of normal behavior; fish swim rapidly to wall of container and swim in place there
	Berde and Corletti (1956-1957), Corletti and Berde (1955)	guppy fish	*Poecilia Platypoecilus reticulatus*	Marked pigment dispersion in melanophores
O. Perciformes F. Centrarchidae	McDonald and Heinstra (1960)	green sunfish	*Lepomis Cyanellus*	Increased fighting by submissive fish without alteration in dominance status
F. Osphronemidae	Abramson and cf Simmons Evans (1954), Evans *et al* (1956), Turner (1956-1956a)	fighting fish	*Betta splendens*	Exaggeration of postures of normal fish; Stuporous state and movements; transient darkening of body pigment; juveniles more sensitive than adults (age effect)

[1] P = Phylum; C = Class; O = Order; F = Family

A. Amphibia O. Urodela F. Salamandridae	Peters and Vowles Salamander (1956)	Triton cristatus		Diminished multifocal eleven or atypical response to stimulating stimulus configurations (very slightly); stiffness in following the electrical activity of the optic cord showed a curtailment discharge of waves of high frequency and amplitude
O. Anura F. Bufonidae	Pengore et al (1956)	toad	Xenopus laevis	Normal pigment concentration in melanophores
C. Aves O. Columbiformes F. Columbidae	Herrman et al (1962)	white domestic pigeon	n.s.	Initial period of sensitivity lowering of response rate in key pecking
	Bloch (1957a, 1957b)	pigeon	n.s.	Reduction in responses to key pecking, decreased sensitivity to light
	Besen & Devine (1963)	pigeon	n.s.	Interference in nesting behavior of male pigeons (increase in time taken to return to nesting); inhibition of perception of nesting
	Siegel (1962)	pigeon	n.s.	Impairment in visual discrimination performance

effects of drugs which under other conditions might be expected to be without serious influence. A comparable situation probably exists in the postpartum woman and her breast fed child as relevant to her concurrent biological pattern. If relatively innoculous drugs pose hazards in such situations then administration of substances, such as LSD, with recognized ill effects, could lead to new and unpredictable risks for both parent and offspring.

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REFERENCES

ABRAMSON, H. A., and L. T. EVANS (1954). Lysergic acid diethylamide (LSD 25): II, Psychobiological effects on the Siamese fighting fish. Science **120** (3128): 990-991.

ABRAMSON, H. A., M. E. JARVIK, and M. W. HIRSCH (1955). Lysergic acid diethylamide (LSD-25). VII. Effect upon two measures of motor performance. Jour. Psychol. **39**: 455-464.

ABRAMSON, H. A., and M. E. JARVIK (1955). Lysergic acid diethylamide (LSD-25): IX. Effect on snails. Jour. Psychol. **40**: 337-340.

ALEXANDER, G. J., D. E. MILES, G. M. GOLD, and R. B. ALEXANDER (1967), LSD: Injection early in pregnancy produces abnormalities in offspring of rats. Science **157** (3787): 459-460.

Appel, J. B., and D. X. Freedman (1965). The relative potencies of psychotomimetic drugs. Life Sci. **4**: 2181-2186.

Auerbach, R., and J. A. Rugowski (1967). Lysergic acid diethylamide: Effect on embryos. Science **157** (3794): 1325-1326.

Baldwin, M., S. A. Lewis, and S. A. Bach (1959). The effects of lysergic acid after cerebral ablation. Neurology **9** (7): 469-474.

Barnett, S. A. (1963). A Study in Behavior: Principles of Ethology and Behavioral Physiology, Displayed Mainly in the Rat. London: Methuen & Co. Ltd.

Berde, B., and A. Cerletti (1956). Effect of LSD and related compounds on melanophores. Helvet. physiol. et. pharmacol. Acta **14**: 325-333.

Berde, B., and A. Cerletti. (1957). Ueber den Angriffspunkt von D-Lysergsäure-diäthylamid und 5-Hydroxytryptamin im Melanophorentest: Zschr. exper. Med. **129**: 149.

Berryman, R., M. E. Jarvik, and J. A. Nevin (1962). Effects of pentobarbital, lysergic acid diethylamide and chloropromazine on matching behavior in the pigeon. Psychopharmacologia **3**: 60-65.

Blough, D. S. (1957). Effects of drugs on visually controlled behavior in pigeons. In: Psychotropic Drugs. S. Garattini and V. Ghetti, eds. Amsterdam, N.Y.: Elsevier Publishing Co., pp. 110-118.

Blough, D. S. (1957a). Some effects of drugs on visual discrimination in the pigeon. Ann. N.Y. Acad. Sci. **66**: 733-739.

Brimblecombe, R. W. (1963). Effects of psychotropic drugs on open-field behavior in rats. Psychopharmacologia **4**: 139-147.

Burgers, A. C. J., W. Leembreis, T. Dominiczak, and G. J. van Oordt (1958). Inhibition of the secretion of inter-medine by d-lysergic acid diethylamide (LSD-25) in the toad, Xenopus laevis. Acta Endocrinol. **29**: 191-200.

Buscaino, G. A., and N. Frougia (1953). Modificazioni biochimiche, elettroencefalografiche, istrochimiche ed istopatologiche, in cani, durante l'intossicazione sperimentale acuta e cronica da dietilamide dell'acid lisergico. Acta neurol (Napoli) **8**: 641.

Cerletti, A., and B. Berde (1955). Die Wirkung von D-Lysergsäure-diathylamid (LSD-25) und 5-Oxytryptamin auf die chromatophoren von Poecilia reticulatus Experientia **11**: 312-313.

Chen, G. M., and J. K. Weston (1960). The analgesic and anesthetic effect of 1-(1-phenylcyclohexyl) piperidine HCL on the monkey. Anesth. Analg. Curr. Res. **39** (2): 132-137.

Clark, R., J. A. Jackson, and J. V. Brady (1962). Drug effects on lever positioning behavior. Science **135** (3509): 1132-1133.

Cohen, M. M. (1968). LSD and chromosomes. Science **4** (a): 76-79.

Cohen, M. M., M. J. Marinello, and N. Back (1967). Chromosome damage in human leukocytes induced by lysergic acid diethylamide, Science **155**(3768): 1417-1419.

Cook, L., and E. Weidley (1957). Behavioral effects of some psychopharmacological agents. Ann. N.Y. Acad. Sci. **66**: 740-752.

Editor (1967). Hazards of drug prescribing in pregnancy. Brit. Med. J. **3**: 220-223.

ELDER, J. T., and J. M. DILLE (1962). An experimental study of the participation of the sympathetic nervous system in the lysergic acid diethylamide reaction in cats. Jour. Pharmacol. Exptl. Therapy. **136**: 162-168.

EVANS, A. H. (1899). Birds. In: The Cambridge Natural History. S. F. Harmer and A. E. Shipley, eds. London: Macmillan and Co. Ltd., vol. 9.

EVANS, L. T., L. H. GERONIMUS, C. KORNETSKY, and H. A. ABRAMSON (1956). Effect of ergot drugs on Betta splendens. Science **123** (3184): 26.

EVARTS, E. V. (1956). Some effects of bufotenine and lysergic acid diethylamide on the monkey. A. M. A. Arch. Neurol. Psychiat. **75** (1): 49-53.

FARRIS, E. J. (1950). The rat as an experimental animal. In: The Care and Breeding of Laboratory Animals. E. J. Farris, ed. New York: John Wiley & Sons, pp. 43-58.

FREEDMAN, D. X., G. K. AGAJANIAN, E. M. ORNITZ, and B. ROSNER (1958). Patterns of tolerance to lysergic acid diehtylamide and mescaline in rats. Science **127** (3307): 1173-1174.

FREEDMAN, D. X., J. B. APPEL, F. R. HARTMAN, and M. E. MOLLINER (1964). Tolerance to behavioral effects of LSD 25 in rat. Jour. Pharmacol. Exp. Ther. **143**: 309-313.

FURSTER, J. M. (1957). Tachistoscopic perception in monkeys. Fed. Proc. **16**: 43.

FURSTER, J. M., and M. VOGT (1956). Some central actions of 5-hydroxytryptamine and various antagonists. Brit. Jour. Pharmacol. **11**: 175-179.

GEBER, W. F. (1967). Congenital malformations induced by mescaline, lysergic acid diethylamide, and bromolysergic acid in the hamster. Science **158** (3798): 265-267.

GELINEO, S., and A. GELINEO (1952). La Température du Vid du rat et sa Signification biologique. Bull. Acad. serbe Sci. math. nat. **4**: 197-210.

GILLETT, E. (1960). Effects of chloropromazine and d-lysergic acid diethylamide on sex behavior of male rats. Proc. Soc. Exp. Biol. Med. **103**: 392-394.

GOGERTY, J. H., and J. M. DILLE (1956). Tolerance to the pyretogenic effects of lysergic acid diethylamide. Jour. Pharmacol. Exptl. Ther. **116**: 450-452.

GOGERTY, J. H., and J. M. DILLE (1956). Pharmacology of d-lysergic acid morpholide. Fed. Proc. **15**: 428.

HALEY, T. J. (1956). Pharmacological effects from drugs injected intracerebrally in unanesthetized animals. Jour. Amer. Pharm. A. (Science Ed.) **45**: 604-607.

HALEY, T. J. (1957). Intracerebral injection of psychotomimetic and psychotherapeutic drugs into conscious mice. Acta. Pharmacol. et toxicol. **13**: 107-112.

HALEY, T. J., and W. G. MCCORMICK (1956). Intra-cerebral injection of LSD-25 in the unanesthetized dog. Fed. Proc. **15**: 433.

HALEY, T. J., and J. RUTSCKMANN (1957). Brain concentrations of LSD-25 (Delysid) after intracerebral or intravenous administration in conscious animals. Experientia **13** (5): 199-200.

HAMILTON, C. L. (1960). Effects of LSD-25 and amphetamine on a running response in the rat. A. M. A. Arch. Gen. Psychiat. **2**: 104-109.

HOFER, A., and H. OSMOND (1967). The Hallicinogens. New York: Academic Press.

HORITA, A., and J. M. DILLE (1954). Pyretogenic effect of lysergic acid diethylamide. Science **120** (3131): 1100-1101.

HORITA, A., and S. CHOROVER (1958). Effects of lysergic acid diethylamise upon certain aspects of memory (delayed alteration) in monkeys. Fed. Proc. **17**: 381.

JONES, D. C. J. KINNELDORF, D. O. RUBADEAU, and T. J. CASTANERA (1953). Relationship between volitional activity and age in the male rat. Amer. Jour. Physiol. **172**: 109-114.

KELLER, D. L., and W. W. UMBREIT (1956). "Permanent" alteration of behavior in mice by chemical and psychological means. Science **124** (3225): 723-724.

KELLER, D. L., and W. W. UMBREIT (1956). Chemically altered "permanent" behavior patterns in fish and their cure by reserpine. Sci. **124** (3218): 407.

KEY, B. J. (1961). The effect of drugs on discrimination and sensory generalization of auditory stimuli in cats. Psychopharmacologia **2**: 352-363.

KEY, B. J., and P. B. BRADLEY (1958). Effect of drugs on conditioning and habituation to arousal stimuli in animals. Nature **182** (4648): 1517-1519.

KHAZAN, N. I., and E. G. SULMAN (1967). The EEG of the olfactory bulb of the rabbit and its relation to psychopharmacological agents. Psychopharmacologia **10** (3): 226-236.

KINDER, E. F. (1927). A study of nest-building activity of the albino rat. Jour. Exper. Zool. **47**: 117-161.

LACEY, J. L. (1956). The evaluation of autonomic responses toward a general solution. Ann. N.Y. Acad. Sci. **67**: 123-163.

LIBERSON, W. T., A. KAFKE, and E. SCHWARTZ (1961). Effects of chlordiazepoxide (librium) and other psychopharmacological agents on "fixated" behavior in rats. Biochem. Pharmacol. **8**: 15-16.

MAFFI, G., and D. CONSTANTINI (1961). Effects of drugs on Timing behavior. Biochem. Pharmacol. **8**: 61-62.

MAHLER, D. J., and F. L. HUMOLLER (1959). Effect of lysergic acid diethylamide and Bufotenine on performance of trained rats. Proc. Soc. Exp. Biol. Med. **102**: 697-701.

MANSOUR, T. E. (1956). Effect of lysergic acid diethylamide, serotonin and related compounds on a parasite trematode, Pasciola hepatica. Fed. Proc. **15**: 454-455.

MANSOUR, T. E. (1957). The effect of lysergic acid diethylamide, 5-hydroxytryptamine, and related compounds on the liver fluke, Fasciola hepatica. Brit. Jour. Pharmacol. **12**: 406-407.

MARRAZZI, A. S. (1962). Synaptic and behavioral correlations of psychotherapeutic and related drug actions. Ann. N.Y. Acad. Sci. **96**: 211-226.

MCQUEEN-WILLIAMS, M. (1935). Maternal behavior in male rats. Science **82**: 67-68.

MCDONALD, A. L., and N. W. HEIMSTRA (1964). Modification of aggressive behavior of green sunfish with d-lysergic acid diethylamide. Jour. Psychol. **57**: 19-23.

MCGAUGH, J. L., L. DEBARON, and V. G. LONGO (1963). Electroencephalographic and behavioral analysis of drug effects on an instrumental reward discrimination in rabbits. Psychopharmacologia **4**: 126-138.

MUNN, N. L. (1950). Handbook of Psychological Research on the Rat: An Introduction to Animal Psychology. Boston: Houghton Mifflin Co.

PETERS, J. J., and A. R. VONDERAHE (1956). Behavior of the salamander under the influence of LSD-25 and Frenquel and accompanying electrical activity of brain and spinal cord. Jour. Nerv. Ment. Dis. **124**: 69-73.

RAY, O. S., and A. S. MARRAZZI (1961). Psychotogen effects on approach and avoidance behavior in rats. Amer. Psychologist. **16**: 453.

RAY, O. S., and A. S. MARRAZZI (1961). A quantifiable behavioral correlate of psychotgen and tranquilizer actions. Science **133** (3465): 1705-1706.

RAY, O. S., and L. W. BIVENS (1966). Performance as a function of drug dose, and level of training. Psychopharma. **10** (2): 103-109.

RICHTER, C. P. (1922). A behavioristic study of the activity of the rat. Comp. Psychol. Monog. **1** (2): 1.

RICHTER, C. P. (1937). Hypophysical control of behavior. Cold Spring Harb. Symp. Quant. Biol. **5**: 258-268.

RICHTER, C. P. (1956). Self-regulatory functions during gestation and lactation. In: Gestation: Transactions of the Second Conference March 8, 9 and 10, 1955. G. A. Villee, ed., Josiah Macy, Jr. Foundation, N.Y., pp. 11-93.

ROMER, A. S. (1966). Vertebrate Paleontology. 3rd. ed. Chicago: University of Chicago Press.

ROSEN, E., and A. JOVINO (1963). Effects of lysergic acid diethylamide on the nesting behavior of male pigeons. Nature **197**: 614-615.

ROSENBLATT, J. S., and D. S. LEHRMAN (1963). Maternal behavior of the laboratory rat. In: Maternal Behvaior in Mammals. H. L. Rheingold, ed., New York: John Wiley & Sons, Inc., 8-57.

ROTHLIN, E. (1957). Lysergic acid diethylamide and related substances. Ann. N.Y. Acad. Sci. **66**: 668-676.

ROTHLIN, E. (1957a). Pharmacology of lysergic acid diethylamide and some related compounds. Jour. Pharm. Pharmacol. **9**: 569-587.

ROTHLIN, E. (1957b). Pharmacology of lysergic acid diethylamide and some of its related compounds. In: Psychotropic Drugs. S. Garattini and V. Ghetti, eds., Amsterdam and New York: Elsevier Publishing Co., pp. 36-47.

ROTHLIN, E., and A. CERLETTI (1952). Uber einige pharmakologische Untersuchungen an Mausen mit congenitaler Drehsucht. Helv. Physiol. Pharmacol. Acta. **10** (3): 319-327.

ROTHLIN, E., A. CERLETTI, H. KONZETT, W. R. SCHALCH, and M. TAESCHLER (1956). Zentrale vegetative LSD-Effekte. Experientia. **12**: 154-155.

SACKLER, A. M., A. S. WELTMAN, and H. OWENS (1966). Endocrine and metabolic effects of lysergic acid diethylamide on female rats. Toxicology and Applied Pharmacol. **9**: 324-330.

SHERMANO, L., and NICKERSON (1956). Effect of ambient temperature on thermal responses to drugs. Fed. Proc. **15**: 462-483.

SHIRLEY, M. (1928). Studies in activity. II. Activity rhythms; age and activity; activity after rest. Jour. Comp. Psychol. **8**: 159-166.

SIEGEL, R. K. (1969). Effects of Cannabis sativa and lysergic acid diethylamide on a visual discrimination task in pigeons. Psychopharmacol. **15**: 1-18.

SIEGEL, S. (1956). Nonparametric Statistics for the Behavioral Sciences. New York: McGraw-Hill Book Company, Inc.

SIMPSON, G. G. (1945). The principles of classification and a classification of mammals. Bull. Amer. Mus. Nat. Hist. **85**: 1-350.

SKAKKEBAEK, N. E., J. PHILIP, and O. J. RAFAELSEN (1968). LSD in mice: Abnormalities in meiotic chromosomes. Science. **160** (3933): 1246-1248.

SNEDECOR, G. W. (1956). Statistical Methods. Iowa State College Press, Ames, Iowa, pp. 45-47.

STURMAN-HULBE, M., and C. P. STONE (1929). Maternal behavior in the albino rat. Jour. Comp. Psychol. **9**: 203-237.

TURNER, W. J. (1956). The effect of lysergic acid diethylamide on Betta splendens. I. Dis. Nerv. System. **17**: 193.

TURNER, W. J. (1956). The effect of lysergic acid diethylamide on Betta splendens. II. Frenquel. Dis. Nerv. System. **17**: 198.

UYENO, E. T. (1966). Inhibition of isolation-induced attack behavior of mice by drugs. Jour. Pharm. Sci. **55**: 215-216.

UYENO, E. T. (1966). Effects of d-lysergic acid diethylamide and 2-brom-lysergic acid diethylamide on dominance behavior of the rat. Int. Jour. Neuropharmacol. **5**: 317-322.

UYENO, E.T. (1968). "Hallucinogenic" compounds and swimming response. Jour. Pharmacol. Exp. Ther. **159**: 216-221.

UYENO, E. T., and W. M. BENSON (1965). Effects of lysergic acid diethylamide on attack behavior of male albino mice. Psychopharmacol. **7**: 20-26.

UYENO, E. T., L. S. OTIS, and C. MITOMA (1968). Behavioral evaluation of hallucinogenic trimethoxy-amphetamines in squirrel monkeys (Saimiri sciureus). Comm. Beh. Biol. **1**: 83-90.

UYENO, E. T., and C. MITOMA (1969). The relative effectiveness of several hallucinogens in disrupting maze performance by rats. Psychopharmacol. **16**: 73-80.

WELTMAN, A. S., and A. M. SACKLER (1966). Metabolic and endocrine effects of lysergic acid diethylamide (LSD-25) on male rats. Jour. Endocrinol. **34**: 81-90.

WEST, O. J., C. M. PIERCE, and W. D. THOMAS (1962). Lysergic acid diethylamide: Its effects on a male Asiatic elephant. Science **138** (3545): 1100-1103.

WIESNER, B. P., and N. M. SHEARD (1933). Maternal Behavior in the Rat. Oliver and Boyd, Edinburgh.

WINTER, C. A., and L. FLATAKER (1956). Effects of lysergic acid diethylamide upon performance of trained rats. Proc. Soc. Exper. Biol. Med. **92**: 285-289.

WINTER, C. A., and L. FLATAKER (1957). Further experiments on the performance of trained rats treated with lysergic acid diethylamide. Jour. Pharmacol. Exptl. Therap. **119**: 194-195.

WITT, P. N. (1951). d-lysergäure-diäthylamid (LSD-25) in Spinnentest. Experientia. **7**: 310-311.

WITT, P. N. (1952). Ein einfachs Prinzip zur Deutung einiger Proportionen im Spinnennetz. Behavior **4**: 172-189.

WOOLEY, D. W. (1955). Production of abnormal (psychotic?) behavior in mice with lysergic acid diethylamide, and its partial prevention with cholinergic drugs and serotonin. Proc. Natl. Acad. Sci. U.S. **41** (6): 338-344.

SHORT COMMUNICATION

EFFECT OF SODIUM GLUTAMATE ON NODULATION AND GROWTH OF SOYBEAN

By V. Iswaran, P. K. Chhonkar, and K. S. Jauhri
Indian Agricultural Research Institute, New Delhi-12, India

This is a study on the effect of sodium glutamate on survival of *Rhizobium japonicum* strain SB-16 on seeds of soybean [*Glycine max* (L.) Merr.] and the subsequent nodulation and growth. Various adhesives like gum arabic, maltose, sugar and methyl cellulose have been used to provide protection for rhizobia resulting in increased survival on seed surface (Date, 1965, 1968; Radcliffe, 1967, Vincent *et al*, 1962).

The method as outlined by Roughley (1966) for slurry inoculation and coating of seed was followed with changes in the ingredients as indicated. The seeds were treated with peat culture containing *R. japonicum* SB-16 by slurry method using 10-per cent sucrose (w/v), 40-per cent gum arabic (w/v), and 40-per cent gum arabic with 1-per cent sodium glutamate as adhesives. Viable cell count on seed surface was determined 1 hour after seed treatment by dilution plate method using yeast extract mannitol agar containing Congo red (Fred *et al*, 1932).

The pot culture experiment was conducted in simple randomized block design with four replications. Each pot contained 5-kg soil and mixed with a basal dressing of 500-mg single superphosphate, 2-mg zinc sulfate; 1-mg manganese; 1-mg ammonium molybdate; and 1-mg borax. Two plants were grown for 6 weeks in each pot. Afterwards the plants were examined for nodulation and fresh and dry weights were recorded.

Table 1.—*Effect of sodium glutamate on survival of Rhizobium japonicum and nodulation and growth of soybeans.*

Treatments	Rhizobia/ seed ($\times 10^4$)	Shoot weight g	Number of nodules	Dry matter g
Uninoculated	—	3.1	20.0	0.8
Inoculated + sucrose	2.5	4.4	68.0	1.2
Inoculated + gum arabic	4.6	5.6	97.0	2.1
Inoculated + gum arabic + sodium glutamate	6.3	7.1	120.0	3.0
C.C. at 5-per cent level		1.4	33.0	0.6

Results in Table 1 show that sodium glutamate increased the survival of *R. japonicum* on seed surface. There was significantly better growth and nodulation over the treatments not receiving sodium glutamate. Maintenance of a high number of viable cells of rhizobia on seed surface at the time of sowing is important in legume inoculation.

REFERENCES

DATE, R. A. (1968). Rhizobial survival on the inoculated legume seed. Trans. 9th Congr. Int. Soil Sci. Soc. Soc. **2**: 75-83.

DATE, R. A., C. BATTHYANY, and C. JAURECHE (1965). Survival of rhizobia on inoculated and pelleted seed. Proc. 9th Intern. Grassld. Congr. 1: 263-269.

FRED, E. B., I. L. BALDWIN, and E. MCCOY (1932). Root nodule bacteria and leguminous plants. Univ. Wisc. Stud. Sci. **5**: 343.

RADCLIFFE, J. C., W. S. MCGUIRE, and N. D. DAWSON (1967). Survival of rhizobia on pelleted seeds of Trifolium subterranium L. Agron. Jour. **59**: 56-58.

ROUGHLEY, R. J., R. A. DATE, and M. H. WALKER (1966). Inoculating and lime pelleting legume seed. Agric. Gaz. NSW **71**: 142-146.

VINCENT, J. M., J. A. THOMPSON, and K. O. DONOVAN (1962). Death of root-nodule bacteria on drying. Aust. Jour. Agri. Res. **13**: 259-270.

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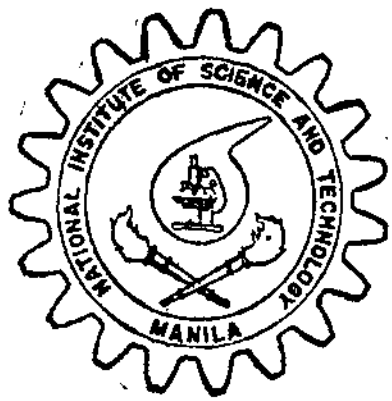

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